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THE EFFECT OF ASPHYXIATION OF THE SPINAL CORD ON PAIN SENSIBILITY

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After application of strychnin (Dusser de Barenne, 1911) or tetanus toxin (Fröhlich and Meyer, 1916) to the spinal cord a marked hyperalgesia of the dermatomes related to the intoxicated segments has been observed. Touching the hyperalgesic parts lightly caused strong defense reactions in the anterior half of the animal. Symptoms of spontaneous pain were also observed.

After recovery of the spinal cord from certain periods of asphyxia, symptoms characteristic of the intoxication with strychnin or tetanus toxin, i.e., high reflex excitability and abolition of reciprocal innervation, have been reported (van Harreveld and Marmont, 1939; van Harreveld, 1939, 1940). The similarity of the effects of strychnin and of asphyxia on reflex excitability made it seem worth while to investigate the effect of asphyxiation of the spinal cord on pain sensibility.

Method. The blood circulation in the caudal part of the spinal cord of cats has been prevented for certain periods of time by forcing Ringer's solution, heated to body temperature, into the dural cavity under a pressure higher than the blood pressure (van Harreveld and Marmont, 1939). In previous experiments the area subjected to increased pressure has been limited by ligating dura and cord in the lower thoracic region. Since for an investigation of the sensibility of the hind limbs the spinal cord had to be left intact, another way to block the free passage between the caudal and cranial part of the dural cavity had to be found. As the spinal cord in the lumbar region is wider than the lumen of the vertebral canal in the adjacent thoracic region, it has been found possible to block up the vertebral canal in the lower thoracic region with the lumbar enlargement. After introducing a hypodermic needle into the caudal part of the dural

cavity between the spinous processes of the 6th and 7th lumbar vertebra, the deeply narcotized animal was brought into a maximally flexed position, by which the cord is pulled cranialwards. If in this position Ringer's solution is forced through the needle under a pressure of 22 to 24 cm. of mercury, the lumbar enlargement is pushed into the narrower thoracic part of the vertebral canal, occluding it. The animal can subsequently be brought in a more normal position, since the pressure will keep the lumbar enlargement in place. The pressure has been maintained for periods of 25 to 65 minutes. The reflex excitability and the sensibility of the hind limbs have been examined frequently after asphyxiation of the cord.

This method has been effective in only about one-third of the animals used. In the others the pressure spread throughout the entire dural cavity, stopping the respiration. This is conceivable since this procedure depends on the individually different relative dimensions of the lumbar enlargements and the thoracic spinal canal.

Results. Asphyxiation of the caudal part of the spinal cord affects the walking movements and the spinal reflexes of the hind limbs as well as the sensibility. The changes in the spinal reflexes can be treated briefly, since they were identical with those reported previously, studied in spinal animals (van Harreveld and Marmont, 1939). Data obtained from six groups of animals in which the intact spinal cord had been asphyxiated for respectively 25, 30, 35, 45, 55 and 65 minutes have been collected in table 1.

In 8 cats the cord was asphyxiated for 25 minutes. The first sign of recovery was the development of tone in the extensor muscles or the return of tendon reflexes (knee- and ankle-jerk) 10 to 30 minutes after the end of asphyxia. The flexion reflex returned after an interval of 50 minutes to 5 hours. In five animals the first reaction in the anterior part of the animal to a strong noxious stimulus (pinching of foot and tail) was seen after 50 to 80 minutes. In one animal (25D) it took 8, in two others (25A and E), 24 hours for this reaction to return. Once developed it remained during the entire period of observation.

In all but one of the cats of this group hyperalgesia was observed. In the pronounced cases (indicated in the table with three crosses) the slightest touch of the fur caused a strong defense reaction in the anterior part of the animal. This hyperalgesia was seen most frequently in the foot, sometimes also in the rest of the leg or in the tail. A few times symptoms have been observed which may indicate spontaneous pain as for instance biting of the foot without apparent cause. In other cats (indicated in the table with two crosses) touching or stroking the fur caused little reaction, but a strong defense reaction in the anterior part could be elicited by lightly rolling the toes of the hind limb between the fingers. This stimulus did not cause any reaction when applied to the

TABLE 1

Interval between the end of asphyxia and the first appearance of reflex activity (knee- or ankle-jerk or extensor tone) and of the flexion reflex. First appearance and eventual later disappearance of the first reaction in the anterior part of the animal to a noxious stimulation of hind leg or tail; of hyperalgesia of leg and tail; of hyperalgesia in the area of the 4th lumbar dermatome and of the walking movements. An asterisk indicates that these phenomena remained during the entire period of observation. The group of cats in which the cord was asphyxiated for 25 minutes is indicated as 25A, B, etc., the group of 30 minute cats as 30A, B, etc. The degree of hyperalgesia is indicated with one, two, or three crosses as is explained in the text. The time in this table is given in hours except in those cases in which the number is followed by the letter d, the time is then expressed in days.

DURATION OF ASPHYXIATION	CAT	FIRST REFLEX (KNEE- OR ANKLE-JERK OR TONE)	FLEXION REFLEX	PAIN SENSIBILITY	HYPERALGESIA IN LEG AND TAIL	HYPERALGESIA IN THE DERMATOME L4	WALKING	PERIOD OF SURVIVAL
minutes								
25	A	0.2	5	24 *		48 *	24 *	25d
	B	0.3	1	1 *	48+ *		1.5 *	5d
	C	0.3	1	1.3 *	48++ 14d	5d *	3 48	21d
	D	0.5	2	8 *	30+ *		8 24	5d
	E	0.3	1.8	24 *	48++ *		3 *	23d
	F	0.3	1	1 *	24+ *		1.3 *	7d
	G	0.2	0.9	0.9 *	2+++ *		2.5 *	34d
	H	0.5	0.8	0.8 *	24+++ *		2 48	29d
30	A	0.3	1.8	1.8 *			2 *	4d
	B	0.2	1.8	1.8 *	11+++ *		3 48	15d
	C	0.5	7	2 *				5d
	D	0.8	3.5	2.8 *				4d
	E	0.2	1.5	0.7 *				47d
35	A	0.2	2	1.3 *				5d
	B	0.8	5	1.2 *		48 *	6.5 12	15d
	C	1.5	8.5	2.5 *				46d
	D	0.7	2	3 *	48++ 5d		3 48	7d
	E	0.3	3.5	6.5 *		3d *	1.5 3d	36d
45	A	1.2		24 *				35d
	B	0.8	3.5	1.5 *	2.5+ 48	3d *		6d
	C	1		1.5 *	6.5++ 24	4d 10d		23d
55	A	2		3 48				21d
	B	2.5		1.2 *	3.5+++ 6			7d
	C	1.4		1.4 *		4d *		42d
65	A	1.4		1.4 36	2+++ 7			15d
	B	1.2		2 10	3++ 7			23d
	C	3		1.2 *	1.8+++ 5	6d *		24d

front leg. In a third group of animals (indicated with one cross in the table) only a careful comparison of the reactions to the same stimulus applied to the front and hind legs revealed the hyperalgesia of the posterior limb. This hyperalgesia appeared a long time (24 to 48 hrs.) after asphyxiation in all cases but one, in which it appeared after two hours. It remained usually during the entire period of observation (up to 34 days), though in most cases diminishing in intensity in the course of time. In one cat (25C) hyperalgesia had practically disappeared after 14 days.

In most of the cats with a marked hyperalgesia there existed a hyperexcitability of the flexion reflex, but there seems to be no direct relation between the degree of hyperalgesia and the excitability of the flexion reflex.

All the animals of this group began to make walking movements with the hind limbs 1.3 to 24 hours after the end of asphyxia. But for an exaggerated extensor tone the walking movements were almost normal in most cats a few hours later. In some cases walking remained that way for the period of observation, in others it became notably poorer 24 to 48 hours later, sometimes even disappearing altogether.

In five cats the cord was asphyxiated for 30 and in five for 35 minutes. The interval between the end of asphyxia and the return of tone and tendon reflexes, of the flexion reflex and of the reaction in the anterior part of the cat to strong pinching of foot or tail was not markedly longer than in the 25 minute group of animals. Once developed the reaction to noxious stimuli remained during the whole period of observation.

In two cats (30B and 35D) hyperalgesia was observed beginning 11 and 48 hours after asphyxia. It diminished in the course of time and could not be demonstrated in one cat (35D) 5 days after asphyxia; in the other it remained. In five cats walking movements were observed in the hind limbs some hours after asphyxia, these movements became very poor some hours later and disappeared entirely in four animals after 12 hours to three days.

In three groups of cats each consisting of three animals the spinal cord was asphyxiated for respectively 45, 55 and 65 minutes. In all the animals extensor tone or tendon reflexes returned though after a somewhat longer interval (up to 3 hrs.) than in the 25 to 35 minute groups of cats. This return was usually temporary. The flexion reflex returned (temporarily) in only one cat (45B) in which the cord had been asphyxiated for 45 minutes. The reaction to strong pinching of foot or tail appeared after an interval which was not markedly longer than after the shorter periods of asphyxia. In three cats (55A, 65A and 65B) the return of sensibility was only temporary; it disappeared after 10 to 48 hours. In the other cats the sensibility to noxious stimuli remained during the entire period of observation.

Six of the cats (45B and C, 55B and 65A, B and C) showed hyperalgesia of the hind limbs and tail, beginning 1.8 to 6.5 hours after the end of

asphyxia. This hyperalgesia was temporary and disappeared usually a few hours later. In two cats (45B and C) it remained for respectively 48 and 24 hours. In some of these cats, especially after 65 minutes of asphyxia, the hyperalgesia was the most pronounced ever observed. No walking movements ever returned to these groups of cats.

In eight cats areas of hyperalgesia developed on the medial side of the thigh and on the back cranial to the root of the tail. Usually the hyperalgesia of the thigh was more pronounced. This localized hyperalgesia developed usually later than the hyperalgesia mentioned before, namely, 2 to 6 days after asphyxiation of the cord. It was found more frequently

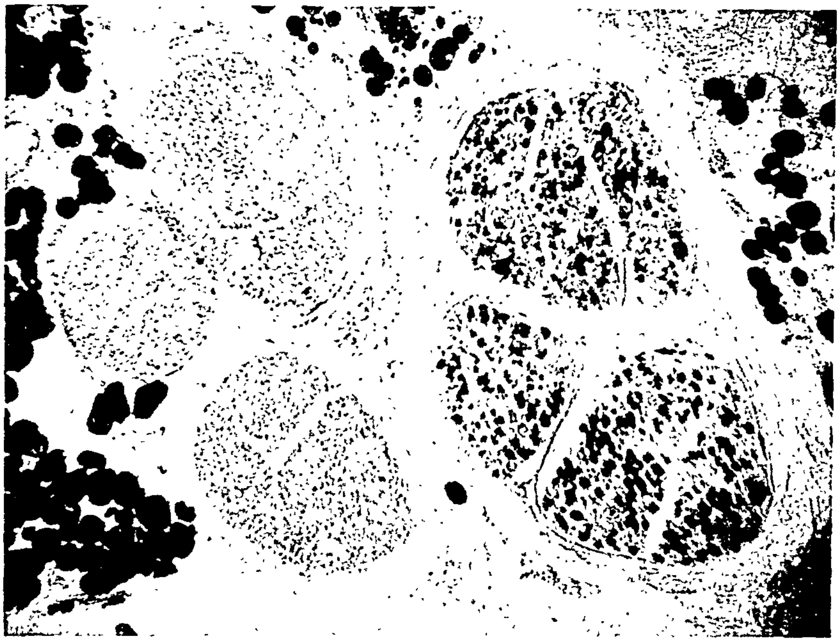

Fig. 1. Dorsal and ventral roots L5 of 55A near the place where the roots penetrate the dura, stained with the Marchi method. The dorsal root (left) does not show any degeneration. An appreciable degeneration is present in the ventral root (right). The large black dots are stained fat cells. Magnification 60 ×.

after the longer periods of asphyxiation, and remained in most cases during the entire period of observation.

Histological examination of the spinal roots. From the cats of which the cord had been asphyxiated for 45 minutes or longer and which had lived afterwards for more than two weeks, the dorsal and ventral roots in the lumbosacral region, stained with the Marchi method, were examined. No degeneration was ever observed in the dorsal roots, whereas a distinct degeneration in the caudal ventral roots was always present (fig. 1). Since even after 65 minutes of asphyxia the dorsal roots did not show degeneration, it can be concluded that asphyxiation had not interfered with the normal sensory supply to the spinal cord.

In two of the five cats thus examined the ventral roots of L5 showed a

strong, in three a moderate degeneration. In the ventral roots of L4 either a slight or no degeneration at all was found. Thus the cranial limit of the area of asphyxiation is situated in the region of the spinal segments L4 and L5.

DISCUSSION. Since the hyperalgesia after asphyxiation remained in many cases until death of the animal (up to 5 weeks after asphyxiation of the cord) this hyperalgesia must be regarded not as a stimulatory effect of asphyxia, but as a release phenomenon, namely, the release from a mechanism which normally prevents impulses set up in the periphery by weak stimuli from reaching the higher centres in such a way that they are experienced as pain. It has to be assumed that this mechanism can be damaged by asphyxia. A similar reasoning with regard to permanent high reflex excitability after asphyxiation of the cord has been given in

TABLE 2

Degeneration in the ventral roots of L6, L5 and L4

Two crosses indicate much, one little degeneration. The animals are marked as in table 1.

DURATION OF ASPHYXIATION	CAT	L6, VENTRAL	L5, VENTRAL	L4, VENTRAL
minutes				
45	A	++	+	–
	C	++	+	+
55	A	++	++	–
65	B	++	+	–
	C	++	++	+

previous papers (van Harreveld and Marmont, 1939; van Harreveld, 1940).

There is a perfect parallel between the development and eventual disappearance of hyperalgesia and that of high extensor tone after asphyxiation of the cord described in a previous paper. Hyperalgesia as well as high extensor tone developed after the shorter periods of asphyxia after a delay of 24 to 48 hours. Once developed they usually remained during the entire period of observation. After the longer periods of asphyxiation both hyperalgesia and high extensor tone returned quicker, but disappeared in a few hours. This course of hyperalgesia and high extensor tone depends presumably, as has been discussed in previous papers, on the greater vulnerability of inhibitory structures and on the ability of neurons, damaged so severely that they will be destroyed shortly, to resume their function temporarily. The latter peculiarity is demonstrated in the present experiments by the walking movements which in many cases returned temporarily after asphyxia. It has to be assumed that after the shorter periods of asphyxia both the structures for the conduction

of the hyperalgetic pain sensation and the structures normally preventing hyperalgesia by blocking this path resume their function; the former permanently, the latter, being more sensitive to asphyxia, temporarily. After a certain time the structure blocking the path used during hyperalgesia stops functioning and a permanent hyperalgesia develops.

After the longer periods of asphyxia the structures normally blocking the path of pain in hyperalgesia are damaged so severely that they will hardly recover at all, whereas the path of the pain sensation recovers temporarily, resulting in a temporary hyperalgesia. Thus the disappearance of hyperalgesia in the latter cases means that the path of the impulses used in hyperalgesia is destroyed and if in these animals pain still can be elicited it has to take another path. In all the cats in which the cord had been asphyxiated for longer periods of time pain reactions could be elicited by strong noxious stimuli after hyperalgesia had disappeared. Thus we have to assume that there are two systems which can conduct impulses resulting in pain sensations. One is normally not apparent, but can under certain conditions (as asphyxiation of the cord) become highly excitable; it is stimulated by stimuli which are not noxious. The second system is stimulated by noxious stimuli. It is more resistant to asphyxia since it was abolished, after returning temporarily, only after the longest periods of asphyxia.

The hyperalgesia localized on the medial side of the thigh and on the back cranial to the root of the tail can be explained in the following way. After the longer periods of asphyxia the cord has been damaged too severely to show a permanent hyperalgesia but at the limits of the area of increased pressure where the asphyxiated cord changes into the normal there must be a region damaged to the right degree to cause this. If this area is wide enough we will observe a permanent hyperalgesia in the related dermatome. According to de Boer's determination of the dermatomes of the cat (1929) both these hyperalgesic spots are part of the dermatome L4 and it has to be assumed that in the spinal segment L4 the circumstances are favorable for the development of this kind of hyperalgesia. The histological examination of the roots showed in agreement with this that the limit of the asphyxiated area is situated in this region.

The hyperalgesia after asphyxiation of the cord is probably not related to the hyperalgesia observed by many authors (see Foerster, 1927) after the severance of the dorsal columns. Foerster has compared the latter form of hyperalgesia with the hyperalgesia after abolition of the epicritic sensibility, which has been explained by Head (1893, 1894, 1896) as a lack of inhibition of the protopathic by the epicritic sensibility. Foerster implies that in transecting the dorsal columns the fibers for the epicritic sensibility are transected. Since these fibers are a direct continuation of the fibres of the dorsal roots and since in the present experiments the latter

were always found to be absolutely intact, there is no reason to believe that their spinal continuation has been damaged by asphyxia.

Fabricius (cited from Foerster, 1927) assumed the existence of a centrifugal tract in the lateral columns which normally inhibits pain sensations. It could be assumed that asphyxia damages spinal structures related to this tract. In Fabricius' conception pain sensations are inhibited by impulses arising in supraspinal structures. It is quite possible, however, that the structure normally preventing hyperalgesia is a strictly spinal one. The existence of a spinal mechanism normally depressing reflex activity (van Harreveld and Marmont, 1939; van Harreveld, 1940) is in favor of this. The assumption of a strictly spinal mechanism readily explains cases of hyperalgesia in which changes in a spinal segment or in the structures related to it result in hyperalgesia in that segment only. Several instances of this kind of hyperalgesia can be given; the hyperalgesia in a dermatome after treatment of the related spinal segment with strychnin, the referred pain and hyperalgesia in dermatomes related to the spinal segments which are connected with a diseased internal organ (Head, 1905), the hyperalgesia after the abolition of the epicritic sensibility (Head, 1893, 1894, 1896). In all these cases hyperalgesia may be caused by influencing the spinal structure which in the normal animal prevents hyperalgesia and which in the present experiments has been damaged by asphyxiation of the cord.

In true neuralgias little significant changes have been found in the first sensory neurone. This is one of the reasons why Frazier, Lewy and Rowe (1937) tried to localize the pathological process underlying neuralgias in the thalamus. The hyperalgesia after asphyxiation of the cord suggests, however, that processes located in the neuraxis may be the cause of this condition.

SUMMARY

Hyperalgesia has been observed in the hind limbs of cats after asphyxiation of the caudal part of the spinal cord. After the shorter periods of asphyxiation (25 min.) this hyperalgesia usually remained for the entire period of observation (up to 5 wks.). After the longer periods of asphyxiation the hyperalgesia was temporary. In the not hyperalgesic parts of the hind limbs or after the disappearance of hyperalgesia in cases in which it occurred temporarily the leg was sensitive to strong noxious stimuli. The long duration of hyperalgesia observed in many cases leads to the conclusion that hyperalgesia is a release phenomenon. The similarity of the course of hyperalgesia and that of high extensor tone after asphyxiation of the cord suggests that both will have to be explained in the same way, namely, by the assumption that asphyxiation damages structures in the cord which normally prevent hyperalgesia or depress extensor tone.

Reasons are presented for the opinion that different structures are involved in the transport of the impulses for pain during hyperalgesia and for the impulses elicited by strong noxious stimuli.

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HEPATIC ACETONE BODY PRODUCTION IN THE DOG DURING FASTING AND FAT FEEDING

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Most of the evidence concerning the production of acetone bodies by the liver has been obtained by indirect methods or from observations on perfused organs or tissue slices. It was believed that data of value might be obtained from analyses of the blood entering and leaving the liver, since ketogenesis appears to be a function of this organ only. We have therefore employed the angiostomy technique, uncomplicated by anesthesia, for a study of hepatic acetone body production, and have chosen fasting or fat feeding as the most nearly "normal" stimulus for ketogenesis.

Our observations have led us to the conclusion that hepatic ketogenesis is an alternative pathway for fat oxidation that is brought into play only when the supply of carbohydrate is inadequate. Unpublished data (Ivy and Crandall) indicate that during fasting there are qualitative changes in the metabolism of lactic acid also. We suggest that such metabolic processes, occurring under conditions of carbohydrate lack and differing qualitatively from those of non-fasted animals, may be referred to as phases of the special metabolism of glucoprivia.

Methods. Normal angiostomized dogs (London cannulae on portal and hepatic veins) were used; the sources of error in this technique have been discussed previously (1). Total blood acetone bodies were determined according to the method of Crandall (2), which is believed to be accurate within 0.1 mgm. per 100 ml. for the low values found in non-fasting animals. All values are expressed as β-hydroxybutyric acid. Determinations of acetone body and glucose concentrations in hepatic, portal, and arterial blood samples have been made in 15 instances on 11 dogs within 24 hours after the last meal and in 25 experiments on 8 dogs that had fasted 2 to 11 days. After fasting, some of the animals were given 100 ml. of olive oil by mouth each day for periods up to 9 days, 13 observations were made on 9 such animals. At the end of the periods of fasting or fat feeding some dogs were given 20 grams of glucose by stomach tube, the hepatic acetone body and glucose outputs being determined be-

fore and at intervals up to $1\frac{1}{2}$ hours after the glucose administration; 6 such experiments on 4 dogs are reported.

The 24 hour output of acetone bodies by the liver was calculated on the assumption that the portal vein contributed $\frac{3}{4}$ and the hepatic artery $\frac{1}{4}$ of the blood reaching the liver; that the concentrations of acetone bodies found in the portal, hepatic, and arterial samples were averages for the day; and that the rate of blood flow through the liver was 28.6 ml. per kilo per minute, which is the average found by Blalock and Mason (3). Because of unknown variations from these assumed averages, the amounts of acetone bodies estimated to be formed by the liver for the 24 hour periods are only rough approximations, and are to be regarded as indicating only the order of magnitude of hepatic ketogenesis. It may be noted, however, that variations in the proportions of blood supplied by the portal vein and hepatic artery would have little effect since the amounts removed by the intestinal tract are small.

The concentration of acetone bodies in the urine was determined by the method of Crandall (2) after removal of interfering substances by precipitation with copper sulfate and calcium hydroxide.

Statistical methods have been applied wherever possible.

RESULTS. Data for animals fed 18 to 24 hours prior to determinations of acetone body concentrations in hepatic, portal, and arterial blood will not be presented individually, since there was little variation from one dog to another. The arterial blood acetone body levels varied from 0.5 to 1.4 mgm. per 100 ml. In no instance did the concentrations in the three samples differ by more than 0.2 mgm., which is regarded as within the limits of error of the method. The mean change produced by the liver in our 15 observations was a retention of 0.01 mgm./100 ml., with a standard error of 0.03. It is apparent that the liver is producing no change in blood acetone bodies that is detectable by the method employed, and that the maximum hepatic output that could have escaped detection is 0.1 mgm./100 ml., which is insignificant.

The data on the fasted (table 1) and fat fed (table 2) animals provide a picture of the development and intensity of ketosis in the dog. It begins gradually after 2 to 4 days of fasting. After 5 to 11 days the liver is adding 1.5 to 8.1 mgm. of ketone bodies to each 100 ml. of blood. The average rate of ketone body liberation for each dog in table 1 on the last day of fasting is 3.1 mgm. per 100 ml., with a standard error of $\pm$ 0.51. The rate for the dogs that had been fasted and then fed fat for three or more days is 6.3 $\pm$ 1.11. The difference between the rates of these two groups is less than three times its standard error, and would be of questionable significance in any case since the effect could be attributed to longer lack of carbohydrate as well as to the fat feeding. If fat feeding does intensify ketosis, which seems probable, the effect is not great.

The utilization of acetone bodies by those tissues drained by the portal vein can be estimated from the data in tables 1 and 2. On the last day of fasting (table 1) it averaged 0.84 mgm. per 100 ml. For the fat fed animals (table 2) it amounted to 1.2 mgm. per 100 ml. (S.E. ± 0.25).

As might be anticipated, there is a positive correlation between rate of hepatic ketogenesis in milligrams per 100 ml. of blood, and the concentration of ketone bodies in the blood. The correlation coefficient (r) for the fasted animals (table 1) is 0.86 ± 0.21; for the whole series of fat fed and fasted dogs r is 0.58 ± 0.17. The fact that these coefficients are significant may be taken as evidence that determination of hepatic output in terms of mgm. per 100 ml. of blood flow indicates the order of magnitude of the hepatic output per unit of time, i.e., that blood flow variations are not

TABLE 1

Hepatic, portal and arterial blood acetone bodies (expressed as milligrams beta-hydroxybutyric acid per 100 ml. of blood) at various intervals during starvation

The urinary acetone body excretion for the corresponding 24 hours is compared with the calculated 24 hour hepatic acetone body formation

DOG	DAYS FASTED	HEPATIC	PORTAL	ART.	HEPATIC OUTPUT	HEPATIC OUTPUT PER 24 HOURS (CALCULATED)	24 HOUR URINARY OUTPUT
					mgm./100 ml.	*grams*	*grams*
1	3	3.3	2.9	3.0	0.4	1.7	
1	8	7.6	3.8	5.0	3.5	15.0	0.238
2	3	3.1	2.6	2.4	0.5	1.9	
2	5	6.3	2.2	4.0	3.6	13.4	
3	3	1.1	1.0	1.0	0.1		
3	6	3.6	1.9	2.5	1.5	8.2	
4	3	2.6	2.1	1.9	0.5	1.8	0.039
4	7	2.8	1.1	1.7	1.6	5.6	
5	6	3.6	1.9	1.9	1.7	9.3	0.089
6	2	0.8	0.8	0.8	0.0		
6	3	2.2	0.9	1.0	1.3	9.4	0.024
6	4	2.0	0.5	1.0	1.4	10.0	0.031
6	6	2.3	1.4	1.7	0.8	5.6	0.090
6	11	8.5	4.1	4.7	4.2	27.4	0.541
7	2	2.5	1.7	2.2	0.7	5.3	0.088
7	3	7.8	2.8	4.3	4.6	34.3	0.341
7	4	10.5	2.8	4.5	7.3	54.2	0.368
7	6	10.6	4.1	6.1	6.0	43.1	0.134
7	11	12.9	7.1	8.1	5.5	37.6	0.239
8	2	1.8	1.6	1.6	0.2		
8	4	2.3	0.9	1.1	1.3	7.1	0.060
8	6	5.7	2.6	3.8	2.8	15.1	0.158
8	9	5.6	2.5	3.4	2.9	15.4	0.105

sufficiently great to impair seriously the value of calculated daily ketone body formation since it is obvious that rate of ketogenesis and rate of utilization determine blood concentration. The amount of ketone bodies formed in 24 hours, according to such calculations, averages 21.6 grams for dogs fasted 4 or more days, and 32.1 grams for those fasted and then fat fed for 3 or more days. The urinary acetone body output for the fasted animals averages 0.187 gram and for the fat fed dogs 0.482 gram. The urinary output is therefore 0.6 per cent of the calculated hepatic production in the fasted and 1.5 per cent in the fat fed dogs. Even though the calculated hepatic output were twice the actual production, the amount of ketone bodies eliminated in the urine would still be an insignificant fraction of the amount formed. One can come to no other conclusion but that at least 95 per cent of the acetone bodies must be oxidized by the tissues. This is in accordance with previous evidence (4, 5).

TABLE 2

Hepatic, portal, and arterial acetone bodies (expressed as milligrams beta-hydroxybutyric acid per 100 ml. of blood) in dogs that have been fasted and then fed 100 ml. of olive or peanut oil per day for several days

The urinary acetone body output per 24 hours is compared with the calculated hepatic acetone body formation for the same 24 hour period

DOG	DAYS FASTED	DAYS FAT FED	HEPATIC	PORTAL	ARTERIAL	HEPATIC OUTPUT	HEPATIC 24 HOUR OUTPUT (CALCULATED)	24 HOUR URINARY OUTPUT
							grams	*grams*
1	8	3	7.5	2.9	5.8	3.9	16.6	0.442
1	8	6	12.0	8.1	10.2	3.4	14.3	0.510
2	5	1	5.6	3.2	4.1	2.2	7.6	
2	5	5	15.2	7.3	8.4	7.5	27.0	0.346
4	7	6	14.4	10.5	12.2	3.5	11.9	0.420
5	6	6	9.6	6.3	7.4	2.7	14.3	0.170
6	11	6	27.0	10.9	11.6	15.9	100.1	0.798
7	11	7	15.3	10.0	9.6	5.4	36.7	0.389
8	9	1	5.7	2.8	3.1	2.8	14.8	0.221
8	9	3	14.8	9.8	10.2	4.9	25.7	0.288
9	6	7	18.6	11.7	12.6	6.8	35.4	0.912
9	6	9	46.8	37.7	40.0	8.5	43.0	0.440
10	6	9	37.4	30.6	31.4	6.6	27.8	0.591

The oral administration of glucose promptly reduces ketogenesis in the liver, as shown in table 3. The rate of decrease in arterial acetone body concentration in the last four experiments varies from 0.20 to 0.26 mgm. per 100 ml. of blood per minute; it cannot be estimated for the first two experiments since the arterial concentration had reached normal levels before the first post-glucose samples were taken. This provides evidence

TABLE 3

The effect of the oral administration of 20 grams of glucose on the hepatic acetone body production of dogs made ketotic by fasting or by fasting followed by daily fat feeding

All results expressed as milligrams beta-hydroxybutyric acid per 100 ml. of blood

DOG	KETOSIS PRODUCED BY	MINUTES AFTER GLUCOSE	ARTERIAL ACETONE BODY CONCENTRATION	HEPATIC ACETONE BODY OUTPUT
6	8 day fast	Before	3.1	3.6
		30	0.7	0.0
		90	0.3	0.2
7	8 day fast	Before	5.8	5.4
		30	0.7	0.3
		90	0.5	0.0
6	11 day fast, then 6 days fat feeding	Before	11.6	15.9
		45	2.5	0.7
		90	1.0	0.0
7	11 day fast, then 7 days fat feeding	Before	9.6	5.4
		30	2.9	1.7
		60	2.5	1.0
8	9 day fast, then 4 days fat feeding	Before	10.2	4.9
		15	6.1	3.4
		30	2.5	2.3
9	6 day fast, then 11 days fat feeding	Before	27.2	6.9
		30	19.4	5.0
		60	13.3	3.5

TABLE 4

Mean hepatic output of glucose and of glucose plus acetone bodies and standard errors in non-fasting and fasting dogs

Data from previous communication included for comparison with those from present series

NUMBER OF EXPERIMENTS	SOURCE OF DATA	DAYS FASTED	HEPATIC GLUCOSE OUTPUT	HEPATIC OUTPUT GLUCOSE PLUS ACETONE BODIES
47	Cherry and Crandall, This Journal **125**: 41, 1939	Less than 1	9.1 ±0.68	
20	All dogs, this series	Less than 1	9.2 ±0.57	
28	All dogs, this series	More than 3	4.7 ±0.41	9.4 ±0.63

for the antiketogenic effect of glucose, but does not disprove the possibility that glucose may also be ketolytic.

Table 4 presents the mean values, with their standard errors, for hepatic

output of glucose and of glucose plus acetone bodies in the present series of animals. For comparison the mean glucose output for a larger series as given by Cherry and Crandall (6) is also shown. Because some of the observations were made in Memphis and others in Chicago the data obtained in the two locations were compared so that any differences in hepatic glucose production due to different laboratory conditions would be revealed. No such differences were found. It is evident that fasting for more than 3 days reduces the amount of glucose liberated by the liver per unit volume of blood almost 50 per cent. When the hepatic acetone body output for each dog is added to its output of glucose, the mean value of the totals is found to be in the same range as the non-fasting output of glucose alone. That is, the statistically significant decrease in hepatic output on fasting is compensated by acetone body production.

DISCUSSION. One of the most salient observations we have made is that the liver does not add acetone bodies to the blood before the second day of fasting in the dog; i.e., ketogenesis does not occur in the normal animal in the post-absorptive state. Since there is good evidence that the liver is the sole site of acetone body formation (7, 8), the absence of ketogenesis correlates well with the low concentration of acetone bodies in the blood of the non-fasting animal. A number of investigators (9, 10, 11, 12, 13) appear to believe that acetone body formation occurs in the non-fasting animal, others have suggested (8, 14, 15, 16) that it is dependent on a decrease in liver glycogen to a critical level. The evidence presented here is believed to be the first direct proof that ketogenesis does not occur when glucose oxidation is not impaired by carbohydrate lack or by some metabolic abnormality.

That fat is oxidized to an appreciable extent in the non-fasting animal can hardly be denied. The lack of formation of acetone bodies under conditions in which fat is being oxidized demonstrates the existence of two alternative routes for fat utilization, one through the ketone bodies and a second which remains unknown but which does not involve ketone body production. Teleologically speaking, the existence of the alternative route which involves acetone body formation argues that when the glucose supply is inadequate the tissues need this type of compound, which is more analogous to glucose than to fat in its proportions of carbon, hydrogen and oxygen.

From the values for daily acetone body output by the liver given in tables 1 and 2 it can be estimated that hepatic ketogenesis can supply up to 57 per cent of the total caloric requirement of the body, and that in the fat fed dogs this process accounts, on the average, for 17 per cent of the total heat production. Such calculations are subject to considerable error and their chief value is to indicate that an appreciable fraction of the total metabolism may be due to acetone body oxidation.

If the hepatic blood flow during fasting does not differ substantially from that of the non-fasting dog, the rate of hepatic glucose production decreases by almost 50 per cent after the third day of starvation. This may explain the decreased blood sugar levels that have been found during fasting in various species (17, 18, 19). We have also observed that the blood sugar concentrations in our fasted dogs may be below what would be considered normal for these animals in the post-absorptive state.

It is of especial interest that the hepatic energy output of the liver of the fasting dog (as glucose plus ketone bodies) is equivalent to its energy output in the non-fasting state (as glucose only.) This again suggests that it it may be essential for the extra-hepatic tissues to receive a certain definite proportion of their fuel in a partially oxidized form.

The following hypothesis is offered in explanation of the occurrence of ketosis: 1, the extra-hepatic tissues require that a certain proportion of their metabolic requirement (presumably varying with the tissue) be supplied by glucose or by acetone bodies; 2, acetone body production therefore decreases the need for glucose production by the liver and conserves the store of carbohydrate and glucose precursors; 3, during fasting or fat feeding the liver maintains a relatively constant output of energy in the form of glucose or glucose plus ketone bodies, the mechanism involved being unknown but the primary stimulus for acetone body production being a lack of preformed carbohydrate and/or an inability to carry out gluconeogenesis at a rate that will meet the total requirement.

Since the completion of these studies, Barnes *et al.* (12) have reported observations on acetone body utilization in the leg of the intact animal. It should be noted that their data are in every respect compatible with the hypothesis here outlined.

We have come to the conclusion that ketogenesis is a special process, in that it does not occur when food is allowed *ad libitum*, and that it appears in response to a failure of the carbohydrate supply caused by fasting or high fat diets.

There is no reason, apparent to us, for regarding ketosis as an abnormal condition except insofar as fasting may be so regarded. Rather it should be looked upon as a mechanism by which the body meets a condition of stress which is likely to occur in the everyday life of most individuals of whatever species.

SUMMARY

Experiments on unanesthetized angiostomized dogs have provided the following information:

1. After 2 to 4 days of fasting the liver begins adding acetone bodies to the blood. Prior to this time no hepatic acetone body production is detectable.

2. The urinary excretion of acetone bodies in our experiments amounts to only a small fraction (probably not more than 5 per cent) of the total amount formed.

3. Assuming that fasting does not produce large changes in the rate of hepatic blood flow, it can be calculated that: *a*, oxidation of acetone bodies may supply up to 50 per cent of the total caloric requirement of the dog after the fifth day of fasting; *b*, after 3 or more days of fasting the hepatic glucose output drops approximately 50 per cent below that observed in the post-absorptive state; *c*, the output of glucose plus acetone bodies during fasting in terms of milligrams or of energy available from their oxidation is equivalent within the limits of error of our determinations to the output of glucose alone in the fasted dog prior to the onset of ketosis.

5. Glucose administered orally is antiketogenic; our data do not permit conclusions concerning a possible ketolytic effect of glucose.

On the basis of these observations it is suggested that there are two alternative pathways for fat oxidation, one through acetone body production in the liver and an alternative pathway not involving ketogenesis. The hypothesis that ketogenesis is a mechanism for supplying the tissues with substances which can be used to replace partially the rôle of glucose in metabolism, and therefore to conserve the carbohydrate reserve and/or to decrease the need for gluconeogenesis, is offered.

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OBSERVATIONS UPON THE PRESSOR SUBSTANCE CAUSING THE RISE IN BLOOD PRESSURE FOLLOWING THE TERMINATION OF TEMPORARY, COMPLETE RENAL ISCHEMIA

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In 1938 Taquini (1), modifying the Goldblatt procedure in dogs, found that reëstablishment of the circulation of kidneys rendered completely ischemic for several hours resulted in a rise in blood pressure. This observation has been confirmed in dogs and extended to cats, rats and guinea pigs (2), although negative results were obtained in rabbits (3).

The hypertension which is due to chronic, partial renal ischemia (Goldblatt) has been the subject of extensive investigation, but few studies have been reported concerning the nature of the rise in blood pressure which occurs upon the termination of temporary, complete renal ischemia (Taquini). It is possible that the factors underlying both types of hypertension are closely related or identical. Certain observations suggest that in chronic, partial renal ischemia the kidney manufactures a pressor substance which is continuously discharged into the general circulation in relatively small amounts, causing chronic hypertension. It may be similarly supposed that in temporary, complete renal ischemia, the same or a related pressor substance accumulates in the kidney and upon restoration of the renal circulation is released suddenly into the blood stream in relatively large amounts, giving rise to acute hypertension.

In 1898 Tigerstedt and Bergmann (4) reported that extracts of normal kidneys contain a pressor substance, which they named renin. It has since been suggested that this substance may be the pressor principle which is responsible for the hypertension resulting from renal ischemia. In 1936 Harrison, Blalock and Mason (5) and Prinzmetal and Friedman (6), working independently, found that the amount of renin in extracts of kidneys of dogs with Goldblatt hypertension is greater than that in control normal kidneys of the same animals.

It is the purpose of this paper to report the following investigations on the nature of the pressor substance which appears to be responsible for the pressor reaction resulting from the termination of complete renal ischemia:

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1. The effect of preliminary injection of piperidomethyl-3-benzodioxane (933F) upon the expected rise in blood pressure resulting from the termination of complete renal ischemia.

2. The effect of preliminary induction of tachyphylaxis to renin upon the expected rise in blood pressure which results from the termination of complete renal ischemia.

3. The comparative pressor effects of extracts of the completely ischemic and control normal kidney of the same animal.

Experiment I—The effect of 933F on the rise in blood pressure following the reëstablishment of circulation in the completely ischemic kidney. 933F is one of many similar compounds synthesized by Fourneau which possess the property of abolishing or reversing the effects of epinephrine injection. 933F has been found to be of distinct value in the study of the nature of pressor substances because of its selective action (7–14), for in doses which reverse the pressor effect of epinephrine and epinephrine-like compounds almost all other epinephrine responses remain unchanged. Katz and Friedberg (15) found that the preliminary injection of 933F in normal and hypertensive dogs abolished or reversed the pressor reaction to epinephrine but did not prevent the pressor effect of renin. These authors concluded that renin is not sympathomimetic in action.

The following experiments were devised to study the effect of 933F on the acute hypertension following the termination of temporary, complete renal ischemia. If this post-ischemic pressor effect is of an epinephrine-like nature, an injection of 933F prior to the restoration of the renal circulation should prevent the rise in blood pressure in the same manner as it does in the instance of epinephrine. The effect of 933F upon the pressor reaction to renin was also investigated.

Method. Each of ten cats was anesthetized with ether, the abdomen opened and one renal pedicle completely occluded by means of a bulldog clamp. The abdomen was then closed and the animal allowed to recover from the anesthetic. Four to six hours later the animal was reanesthetized with nembutal intraperitoneally, the abdomen reopened through the previous incision and the clamp exposed. The carotid artery was cannulated and the blood pressure recorded in the usual manner. Epinephrine (0.025 mgm.) was injected intravenously. After the blood pressure had returned to the pre-injection level, 933F in dosage of 1.5–3 mgm. per kilogram was similarly administered. The blood pressure was again allowed to return to its original level and the previous injection of epinephrine repeated. The clamp occluding the renal pedicle was then released after a constant level in blood pressure had been established. When the blood pressure had again returned to a constant level following the release of the clamp, 0.025 mgm. of epinephrine was injected, this time into the substance of the kidney. In four instances additional injections of 1 cc. of renin (prepared from the kidneys of cats by the method of Pickering and Prinzmetal (16)) were made into the external jugular vein before and after the injection of 933F. In a fifth animal an injection of 1 cc. of renin was given only after the administration of 933F.

RESULTS. Preliminary injection of epinephrine caused a characteristic pressor response in each instance. Following the injection of 933F the pressor effect of epinephrine was reversed. Removal of the clamp occluding the renal pedicle was followed by a rise in blood pressure in all but one instance, the average rise in blood pressure for all ten animals being 30 mm. of Hg. The injection of epinephrine into the substance of the kidney after the pedicle had been released resulted in a lowering of the blood pressure in each instance, the average fall being 36 mm. of Hg. This proved that the circulation to the kidney had been reëstablished and that the effect of 933F was still operative. The injection of renin, both before and after the administration of 933F, caused an elevation in blood pressure

TABLE 1

The effect of 933F on the pressor response following reëstablishment of the circulation of completely ischemic kidneys of cats

ANIMAL NUMBER	BLOOD PRESSURE RESPONSE TO 0.025 MGM. EPINEPHRINE	BLOOD PRESSURE RESPONSE TO RENIN	BLOOD PRESSURE RESPONSE TO SECOND EPINEPHRINE	BLOOD PRESSURE RESPONSE TO SECOND RENIN	BLOOD PRESSURE RESPONSE TO REËSTABLISHMENT OF CIRCULATION OF ISCHEMIC KIDNEY
1	+50		−30		−10
2	+26		−40		+6
3	+44	+18	−54	+14	+12
4	+56	+16	−60	+34	+12
5	+60	+26	−60	+24	+104
6	+30		−72		+4
7	+50		−34		+42
8	+60	+40	−40	+46	+20
9	+64		−38	+20	+16
10	+100		−48		+52
Averages...	+54	+25	−47	+26	+30

in all animals so treated; the average rise before 933F, in four animals, was 25 mm. of Hg, and the average rise after 933F, in five animals, was 26 mm. of Hg (fig. 1, table 1).

Experiment II—The effect on the blood pressure of reëstablishment of the circulation in the completely ischemic kidney of animals rendered tachyphylactic to renin. Tigerstedt and Bergmann (4) found that repeated injections of renin resulted in progressively diminishing pressor responses, a phenomenon known as tachyphylaxis. The nature of this phenomenon has not been fully elucidated, although Page and Helmer (17) believe that renin-activator, a substance present in normal blood and necessary for the activation of renin, may be depleted by repeated injections of renin, thus causing diminishing responses.

The following experiment was devised to determine whether or not the

hypertension which results from the reëstablishment of the circulation in the completely ischemic kidney is prevented in animals rendered tachyphylactic to renin. A significant reduction or abolition of the pressor response would suggest that the post-ischemic hypertension is due to renin or a renin-like substance.

Method. Twenty-two cats were anesthetized with ether. In fourteen animals one renal pedicle was completely occluded by means of a bulldog clamp. In the remaining eight, both renal pedicles were similarly occluded. Four to six hours later each animal was reanesthetized with nembutal intraperitoneally, and the blood pressure recorded as before. The abdomen was then reopened and the clamp or clamps exposed. In eleven of the twenty-two animals, repeated injections of renin

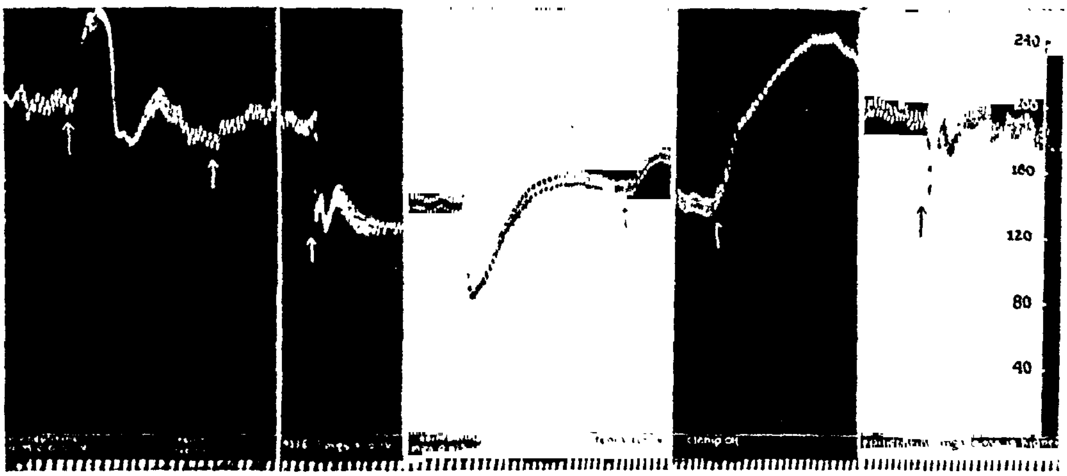

Fig. 1. Effect of previously administered 933F on the expected rise in blood pressure following the reëstablishment of circulation in the ischemic kidney of the cat. At first arrow 0.025 mgm. of epinephrine i. v. At second arrow 1 cc. of renin i. v. At third arrow i. v. 933F in dosage of 3 mgm. per kilogram. At fourth arrow 0.025 mgm. of epinephrine i. v. At fifth arrow 1 cc. of renin i. v. At sixth arrow release of clamp completely occluding renal pedicle for five hours. At seventh arrow 0.025 mgm. of epinephrine injected into substance of kidney with reëstablished circulation. Blood pressure in mm. Hg. Time marker 15 seconds.

were administered in the external jugular vein. Except in one instance, in which purified renin prepared by the method of Helmer and Page (18) was used, the renin injected was prepared from the kidneys of cats by the method of Pickering and Prinzmetal (16). It required five to twenty-one injections for the induction of tachyphylaxis. At this point, when a constant level in blood pressure had been established, the clamp (3 animals) or clamps (8 animals) occluding the renal pedicle or pedicles were released and changes in blood pressure observed. In the eleven remaining animals, which served as controls, only one pedicle was clamped. In these no renin was injected, and the occluding clamp was released as soon as a constant level in blood pressure was obtained.

Results. In eight of the eleven experiments in which induction of tachyphylaxis preceded restoration of the circulation in the ischemic kidney, no rise in blood pressure followed the removal of the clamp or clamps

occluding the renal pedicles; small rises in blood pressure (8, 10 and 10 mm. of Hg, respectively) were observed in the three remaining animals. The

TABLE 2A

Effect of reëstablishment of circulation of completely ischemic kidneys of cats following induction of tachyphylaxis by repeated injections of renin

ANIMAL NUMBER	NUMBER OF INJECTIONS OF RENIN	CIRCULATION REËSTABLISHED IN 1 OR 2 KIDNEYS	BLOOD PRESSURE BEFORE RE-ESTABLISHMENT OF CIRCULATION	BLOOD PRESSURE FOLLOWING RE-ESTABLISHMENT OF CIRCULATION	NET RISE IN BLOOD PRESSURE
			mm. Hg	*mm.* Hg	*mm.* Hg
1	6	1	214	214	0
2	21	1	170	170	0
3	11	1	176	186	10
4	16	2	222	222	0
5	5	2	168	168	0
6	13	2	188	188	0
7	9	2	144	154	10
8	11	2	206	206	0
9	12	2	190	190	0
10	12	2	198	206	8
11	9	2	206	206	0
Averages...	11		189	191.5	2.5

TABLE 2B

Effect of reëstablishment of circulation of completely ischemic kidneys of cats

ANIMAL NUMBER	CIRCULATION REËSTABLISHED IN 1 OR 2 KIDNEYS	BLOOD PRESSURE BEFORE RE-ESTABLISHMENT OF CIRCULATION	BLOOD PRESSURE FOLLOWING RE-ESTABLISHMENT OF CIRCULATION	NET RISE IN BLOOD PRESSURE
		mm. Hg	*mm.* Hg	*mm.* Hg
1	1	144	172	48
2	1	127	182	55
3	1	162	242	80
4	1	128	168	40
5	1	144	184	40
6	1	140	176	30
7	1	143	170	27
8	1	125	148	23
9	1	166	186	20
10	1	132	156	24
11	1	160	188	28
Averages.....		143	181	39

average rise in blood pressure in these eleven tachyphylactic animals was 2.5 mm. of Hg (fig. 2a, table 2a). In the eleven control animals a significant elevation in blood pressure was noted in each instance upon release of

the clamp occluding the renal pedicle, the average rise being 39 mm. of Hg (fig. 2b, table 2b).

Experiment III—Comparison of the pressor effects of extracts of completely ischemic kidneys and control normal kidneys of the same animals. It has been found that the amount of renin is greater in partially ischemic kidneys of hypertensive dogs than in control normal kidneys of the same

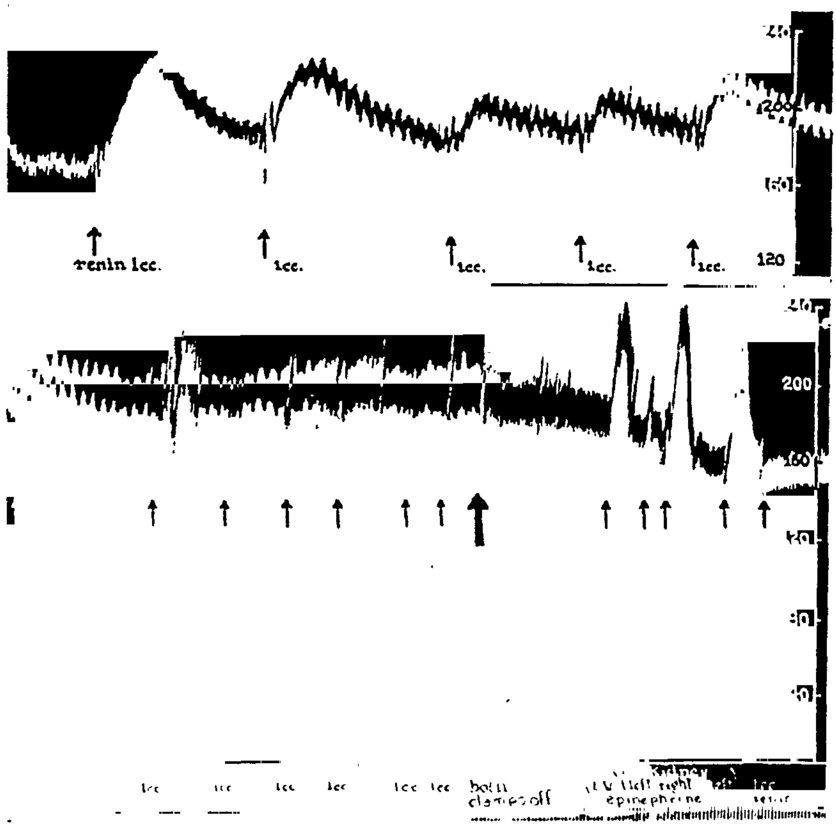

Fig. 2a. Effect on blood pressure of reëstablishment of circulation in both ischemic kidneys of a cat previously rendered tachyphylactic to renin. First twelve small arrows indicate intravenous injections of 1 cc. of renin. Clamps completely occluding both renal pedicles for five hours released at large arrow. Following four small arrows indicate injections of 0.025 mgm. of epinephrine, first intravenously, then into substance of left, right, and again left kidney; 1 cc. of renin injected intravenously at last small arrow. Blood pressure in mm. Hg. Time marker 15 seconds.

animals (5, 6). It is therefore important to compare the amount of renin in kidneys rendered completely ischemic with that in control normal kidneys of the same animals. For this reason the following experiment was performed:

Method. Ether anesthesia was employed in each of eleven cats. One renal pedicle was occluded. Four to six hours later the cat was re-anesthetized with nembutal intraperitoneally, and both the normal and ischemic kidneys removed.

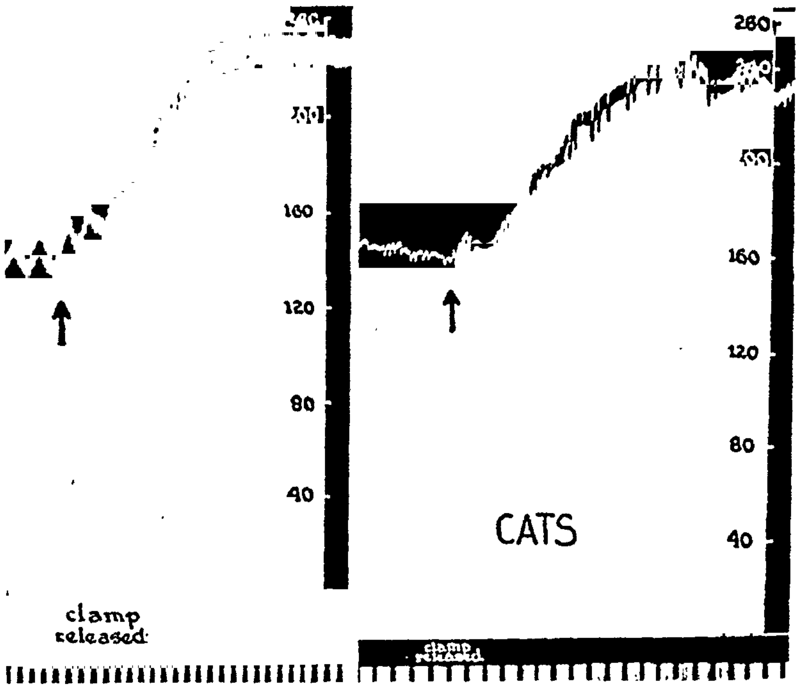

Fig. 2b. Effect on blood pressure of reëstablishment of circulation in one ischemic kidney of a non-tachyphylactic cat. Clamp completely occluding renal pedicle for five hours released at arrow. Blood pressure in mm. Hg. Time marker 15 seconds.

TABLE 3

Assay of renin in completely ischemic and control normal kidneys of cats

ANIMAL NUMBER	PRESSOR EFFECT OF RENIN EXTRACTED FROM NORMAL KIDNEY	PRESSOR EFFECT OF RENIN EXTRACTED FROM ISCHEMIC KIDNEY
	mm. Hg	*mm.* Hg
1	4	16
2	14	23
3	15	27
4	18	38
5	25	42
6	24	24
7	−2	14
8	−1	10
9	0	12
10	0	15
11	−2	13
Averages..............	8.8 (±3.8)	21.3 (±3.3)

The difference of the averages is 12.5 (±4.9).

The average of the differences found in individual pairs of experiments is 12.6 (±1.7), an undoubtedly significant difference.

Extracts were prepared from both kidneys and assayed for their pressor effects upon unanesthetized rabbits by methods previously described (16).

Results. Each pair of extracts consisted of the extract of ischemic kidney and that of the opposite normal control kidney. In ten of the eleven pairs the pressor effect of the extract of ischemic kidney was greater than that of the extract of normal kidney, and in the eleventh pair the pressor effect of each extract was the same. The average rise in blood pressure for the eleven extracts of the ischemic kidneys was 21 mm. of Hg, compared to 8 mm. of Hg, for the eleven extracts of the normal kidneys (table 3).

Discussion. In this communication certain observations are reported upon the pressor reaction which follows the termination of temporary, complete renal ischemia, in an effort to throw light upon the nature of the hypothetical pressor substance which is presumably formed in the ischemic kidney.

The first experiment demonstrated that a previous injection of 933F reversed the pressor effect of epinephrine, but failed to abolish that following reëstablishment of the circulation in the completely ischemic kidney. It is therefore concluded that the substance responsible for the rise in blood pressure following the termination of complete renal ischemia is not epinephrine or an epinephrine-like substance.

In the second experiment it was shown that the rise in blood pressure ordinarily following termination of complete renal ischemia was abolished or markedly diminished in animals previously rendered tachyphylactic to renin. These observations prove that the substance causing the rise in blood pressure is renin or a principle having similar properties of tachyphylaxis. Until more is known concerning the nature of tachyphylaxis, however, no further conclusions can be drawn.

The finding of increased amounts of renin in completely ischemic kidneys of cats recalls previous observations showing the amount of renin to be increased in partially ischemic kidneys of hypertensive dogs. These observations would seem to indicate that as a result of total renal ischemia, renin is either formed in greater quantities than in normal control kidneys, or becomes more readily available for extraction. In this connection, reference may be made to recent studies by Goormaghtigh (19) who noted hypertrophy and hyperplasia of certain cells in the preglomerular arterioles of ischemic kidneys. It is possible that renin is formed in these cells and that their increased activity in renal ischemia may indicate augmented production of this substance.

CONCLUSIONS

1. The previous injection of piperidomethyl-3-benzodioxane (933F) does not prevent the rise in blood pressure which results from the reëstablish-

ment of the circulation in the completely ischemic kidney, proving that the substance responsible for this rise is not of an epinephrine-like nature.

2. The pressor reaction which follows the termination of complete renal ischemia is greatly reduced or abolished in animals rendered tachyphylactic to renin. This proves that the substance causing the rise in blood pressure is renin or a pressor principle having similar properties of tachyphylaxis.

3. The pressor effect of extracts of completely ischemic kidneys is greater than that of extracts of control normal kidneys.

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THE SUBSTANCE OF THE ANTERIOR PITUITARY GLAND WHICH INCREASES LIVER FAT

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It was observed (Best and Campbell, 1936, 1938) that certain extracts from the anterior pituitary gland produced a great increase in the liver fat of fasting animals. Since that time we have been interested in the purification of the active material. For this purpose a means of assaying the so-called "liver-fat" activity was developed (Campbell, 1938). The extent to which this activity is possessed by the various fractions of the anterior pituitary gland extracts and the properties of the extracts containing this active substance in highest concentration are described in this paper.

METHODS. The method of assaying the liver fat increasing activity (L.F.A.) of the anterior pituitary extracts has already been described. Ketogenic activity was determined by a method which may be outlined as follows. Male rats of 100 to 200 grams were fasted in Hopkins metabolism cages for 24 hours and then given a single injection of extract. The urinary excretion of total acetone bodies during the following 24 hours was determined by the method of Van Slyke (1917). The response produced by the unknown extract (in 5 rats) was compared with the responses produced by two doses of the standard anterior pituitary preparation (A.P.P.). After a rest period of 10 to 14 days the rats were used again. The responses apparently become more uniform after the animals have been used for testing about 3 times. Shipley and Long (1938) question the value of results obtained from the determination of urinary ketones in rats. Others, however (Gray, 1938, Neufeld and Collip, 1938), have used modifications of this method and for the purposes of assay under standard conditions we have found it to be satisfactory.

Diabetogenic activity was tested on normal young male dogs of about 10 kgm. weight. These animals were given a diet of 400 gram lean meat and 40 gram sucrose daily with an adequate supply of vitamins. Daily subcutaneous injections of extract were given. Diabetogenic activity was indicated by a rise in blood sugar to about 200 mgm. per cent in about 3 days and the appearance of glycosuria at the same time or somewhat later.

Prolactin activity was determined according to the method of Riddle,

Bates and Dykshorn (1933). Over a 4-day period the extracts were injected daily into the breast muscle of pigeons; the proliferation of the crop glands was then determined by weight and by noting the amount of crop milk which was present. The "local intradermal" crop gland response method (Lyons, 1937) has also been used. Melanophore-expanding activity was determined by injection of the extract into the dorsal lymph sacs of frogs which were exposed to light. The activity was estimated by the degree of darkening produced by the injections.

The method of preparing the A.P.P. has been described by Best and Campbell (1936) in their experiments on rats. Samples of this preparation, kept as a dry powder at 7°C., have shown no demonstrable loss of potency in 5 years. The prolactin and follicle-stimulating hormone (F.S.H.) were prepared according to the method of Bates and Riddle

Preparation no. 222

100 grams dry defatted anterior pituitary glands
1500 cc. water
150 cc. N/1 HCl
Centrifuged

- Residue
  Reëxtracted
- Combined supernatants
  to pH 5.2 with NaOH
  - Precipitate
    Fraction B
  - Supernatant
    Alcohol to 50%
    - Precipitate
      Fraction C
      yield 3.9%
    - Filtrate
      4 vols. alcohol
      1 vol. ether
      - Precipitate
        Fraction F
        yield 3.9%
      - Supernatant
        Discarded

Fig. 1

(1936). The fractions referred to as mammotropic and adrenocorticotropic by Lyons (1937) were also prepared.

The extract numbered 222F was prepared from dry defatted anterior pituitary glands. The fresh glands were treated with acetone and ether to yield a dry light-colored product which was then finely ground. This dry material was extracted (fig. 1) with aqueous HCl by mechanical stirring for 3 hours. The insoluble material was separated by centrifuging and reëxtracted. The combined filtrates were brought to pH 4.5 and the precipitate removed. To the filtrate an equal volume of 95 per cent ethyl alcohol was added slowly while stirring. The precipitate was removed and to the filtrate two volumes of alcohol, each equal to the first, and 1 volume of ether were added. The precipitate (no. 222F) was collected by decanting and centrifuging, and was dried with alcohol and ether. The powder was dissolved in dilute NaOH, brought to pH 8 and the insoluble material removed by centrifuging.

For the preparation of the globulin extract the anterior lobes of the pituitary glands were dissected immediately after removal from the animal and frozen in CO_2 snow. The glands were then minced and extracted as outlined in figure 2 at temperatures not exceeding 7°C. The "diabetogenic" activity is apparently easily lost and the extract was therefore stored at 4°C. for periods not exceeding 1 week. All these extracts were prepared from the pituitary glands of cattle.

Results. *Relation of liver fat activity to prolactin.* In our hands the method of Bates and Riddle (1936) has yielded the most satisfactory

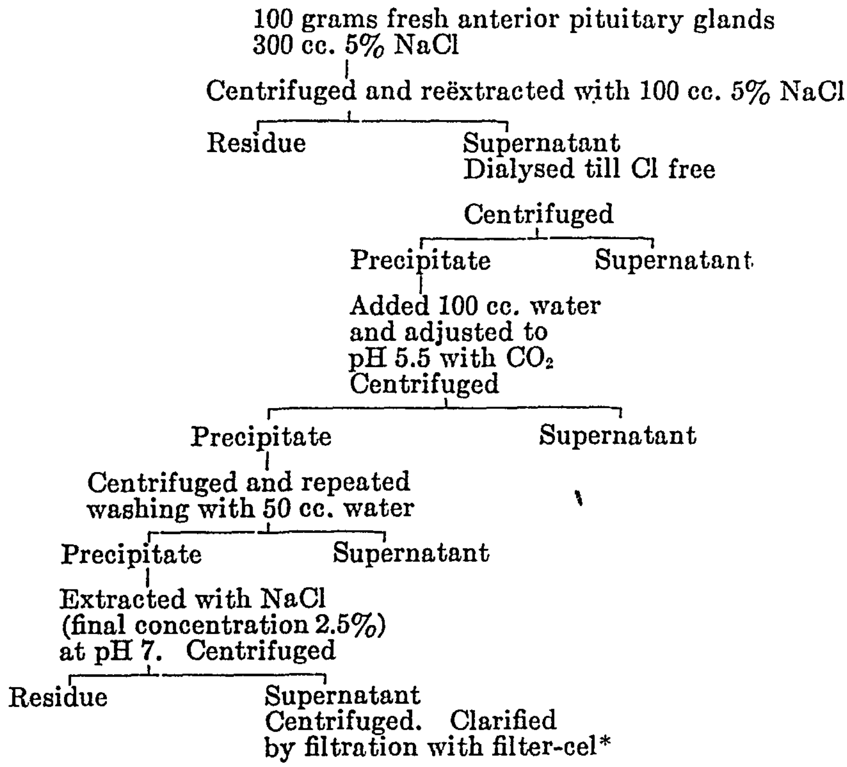

Fig. 2

* A filter aid prepared by the Johns-Manville Co.

preparations of prolactin. Four separate preparations were all highly active in producing proliferation of the crop gland in pigeons, while the A.P.P. produced only a slight crop gland response. The prolactin preparations have been tested for L.F.A. on 7 occasions and each time were found to be less active than the A.P.P. (table 1). The average of all the tests showed that the prolactin preparations had 28 per cent of the activity of the standard. Attempts were made to free the prolactin of all L.F.A. by repeated precipitation of the prolactin from dilute alkaline aqueous solution by the addition of dilute hydrochloric acid and also acetic acid to pH 4.85. A portion of the L.F.A., which remained in the filtrates, was

thus removed from the prolactin precipitate but the separation was not complete.

The F.S.H. fraction of Bates and Riddle (1936), on the other hand, showed very high L.F.A. (250 per cent) and negative prolactin activity when compared with the standard preparation.

The fractions which have been termed mammotropic and adrenocorticotropic by Lyons (1937) were prepared, but since the latter fraction had considerable crop gland stimulating potency the separation was not as complete as the author indicates. Three tests on each of these fractions showed that they contained less L.F.A. than the A.P.P. The average for the mammotropic fraction was 42 per cent and for the adrenocorticotropic fraction 77 per cent. The mammotropic fraction produced much greater crop gland proliferation than the A.P.P.

TABLE 1

PREPARATION	LIVER FAT INCREASING ACTIVITY	KETOGENIC ACTIVITY	PROLACTIN	DIABETOGENIC ACTIVITY
	per cent	*per cent*		
Standard preparation	100	100	+	Slightly active
Prolactin (acc. to Bates and Riddle, 1936)	28	9	+++++	Negative
Mammotropic (acc. to Lyons, 1937)	42		++++	
F.S.H. fraction (acc. to Bates and Riddle, 1936)	250		No activity	
Adrenocorticotropic fraction (acc. to Lyons, 1937)	77		++	
Fraction # 222F	330	17	No activity	
Globulin extract	22–100	120–500	+++++	Highly active

A sample of prolactin prepared by Doctor White of Yale University was tested for L.F.A. It was estimated (by a single test) that this material contained 30 per cent of the activity of the A.P.P. These results indicate that prolactin and L.F.A. are separable.

Relation of liver fat activity to ketogenic activity. The amounts of ketogenic and L.F.A. which were present in the globulin fraction varied considerably from one preparation to another, although the same method of preparation was used. In 4 preparations the ketogenic activity averaged 142 per cent and the L.F.A. 46 per cent (table 1). It thus appeared that in this fraction, as compared to the A.P.P., the ketogenic and L.F.A. did not occur in constant proportions. The ketogenic activity of 8 other globulin preparations was higher than in the former, averaging 319 per cent, but in these L.F.A. was not determined.

The preparation no. 222F showed the most striking difference between ketogenic and L.F.A. The L.F.A. (3 tests) averaged 330 per cent while the ketogenic activity (4 tests) averaged only 17 per cent. It appears that these two forms of activity are not necessarily associated.

Relation of liver fat activity to melanophore-expanding activity. Determination of the liver fat and melanophore-expanding activities in extracts from different parts of the pituitary gland (pars anterior, posterior, intermedia, tuberalis and pituitary colloid) showed that there was no correspondence between these activities. The melanophore-expanding activity per gram of tissue was greatest in the posterior lobe and least in the anterior, while the L.F.A. was greatest in the anterior and least in the posterior lobe extracts.

Relation of ketogenic activity to diabetogenic activity. The globulin fraction of the anterior pituitary given subcutaneously has been found to be highly active in producing transient diabetes in dogs, and in three cases permanent diabetes. This extract also produced a rapid fall in insulin content of the pancreas (Best, Campbell and Haist, 1939). In doses of 12 to 27 mgm. per kgm. per day (the upper limit being in excess of that required to produce an effect) the globulin produced glycosuria. Out of 23 dogs, 20 showed distinct glycosuria and 3 gave no response. This fraction is therefore high in diabetogenic activity. Although this test was not quantitative, it appeared that diabetogenic activity was associated with ketogenic activity as repeated tests on the globulin fraction indicated that a parallelism existed between them.

Relation of prolactin to diabetogenic activity. The "globulin" fraction was the only one of all those tested which produced hyperglycemia and glycosuria with any regularity in normal dogs. This extract was also highly active in producing crop gland proliferation so that in this case diabetogenic activity has not been separated from prolactin activity.

The diabetogenic activity of prolactin prepared by the method of Bates and Riddle has been tested in 3 normal dogs given large doses of extract. The prolactin produced good proliferation of the crop gland and was estimated to contain 1 to 2 units (Riddle) per mgm. Dog 1, a male of 9.1 kgm., was injected daily for 17 days with an average of 77 mgm. prolactin per kgm. No significant rise in urinary sugar occurred except on the 7th day when 3.85 grams were excreted. Dog 2, a male of 9.6 kgm., received 13 injections averaging 40 mgm. per kgm. per day but no rise in urinary sugar occurred. Dog 3, a female of 7.5 kgm., received 16 injections of 97 mgm. per kgm. per day. The mammary glands hypertrophied and lactation ensued about the 10th day but no change in sugar excretion was observed.

These experiments indicate that prolactin is not necessarily associated with diabetogenic activity and therefore support the findings of Young (1938), Shipley and Long (1938) and Houssay and Biasotti (1938).

Relation of prolactin to ketogenic activity. The ketogenic activity of the prolactin prepared according to Bates and Riddle was low (table 1) when compared with the A.P.P. Since the prolactin activity of the A.P.P. was low it appears that ketogenic activity is separable from prolactin activity.

Nature of the material which increases liver fat. The substance which increases liver fat is soluble in water in the presence of M/20 NaCl at pH 7. The region of minimum solubility occurs about pH 4.8 (reaction adjusted with HCl) at which point most of the active substance is precipitated. It is soluble in 65 per cent ethyl alcohol at pH 9 and at pH 3, but is insoluble at pH 5 to 7.

TABLE 2

Liver fat determined 7 hours following the administration of extract to fasting female mice

Series A. A.P.P. incubated for 4 hours at 37°C., pH 2.5 with pepsin.
Series B. A.P.P. incubated for 4 hours at 37°C., pH 2.5 with boiled pepsin.
Series C. Saline.
Series D. A.P.P. incubated in phosphate buffer for 3½ hours at 37°C., pH 8.2 with trypsin.
Series E. A.P.P. incubated in phosphate buffer for 3½ hours at 37°C., pH 8.2 with boiled trypsin.
Series F. Saline.

SERIES	DOSAGE OF A.P.P. PER MOUSE	NUMBER OF MICE	AVERAGE WEIGHT	LIVER FAT		
				Per cent	Mgm. per 100 grams body weight	Increase over control
	mgm.		*grams*			
A	7	20	18	5.69	377.	9.
B	7	20	18	9.00	709	441
C	0	10	19	5.02	368	0
D	10	20	17	4.62	278	24
E	10	20	17	12.4	762	510
F	0	10	17	4.47	252	0

We have been unable to produce definite gains in the activity of fractions which increase liver fat by fractionation with ammonium sulphate. This has been investigated by step-wise increases in the concentration of ammonium sulphate, or solution of material precipitated by higher concentrations with lower concentrations of ammonium sulphate. The active material is, however, completely precipitated by 60 per cent of saturated ammonium sulphate from pH 7 to 5.

Experiments (table 2) have shown that the L.F.A. is rapidly destroyed by digestion with pepsin and trypsin at 37°C. for 4 and 3½ hours respectively. The active material is therefore protein-like or is combined with protein material.

The curves for the inactivation of L.F.A. at 58°C. (fig. 3) show that the point of maximum stability is in the neighborhood of pH 3.5 and also that the substance is fairly heat-stable. Tests have shown that the ketogenic substance is most stable about pH 8.0 and is rapidly destroyed at pH 3.5

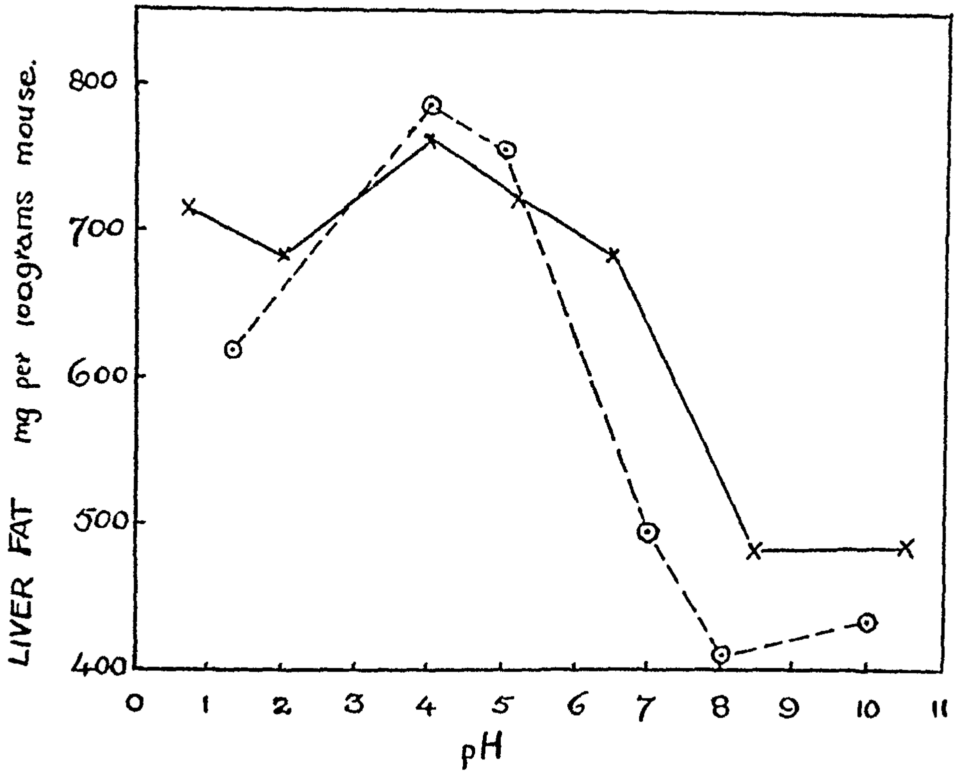

Fig. 3. Curve X——X Solutions heated at 58°C. for 21 hours. With unheated solution liver fat = 815. Curve ⊙- - -⊙ Solutions heated at 58°C. for 22 hours. With unheated solution liver fat = 968.

TABLE 3

Ketone body excretion—mgm. per rat, 5 rats per group

Temperature at which the solutions were heated = 58°C.

	TIME OF HEATING		
	30 minutes	42 minutes	110 minutes
pH 3.5	2.1	1.6	No response
pH 6.5	4.1	16.7	No response
pH 8.0	8.9	37.8	2.0

(table 3). In addition, the ketogenic substance appears to be more heat labile than the liver fat increasing substance since the former is almost completely inactivated in 2 hours at 58°C. and pH 8.0. The latter, however, still retains a large part of its activity after 22 hours at 58°C. and pH 3.5.

DISCUSSION. It is apparent that the prolactin activity of anterior pituitary extracts does not parallel liver fat activity (L.F.A.). However, none of the prolactin preparations tested were entirely free from L.F.A. Although this is probably the result of incomplete purification it is necessary to consider the possibilty that pure prolactin may be able to increase liver fat, although in much less degree than other preparations. It has also been kept in mind that the procedures of fractionation may alter the properties and the activities of the native proteins present in the anterior pituitary gland. Bates and Riddle (1936) found that prolactin is most stable to heat at pH 8.0, while we have shown that L.F.A. is most stable at pH 3.5. This is another indication that these substances are distinct.

It was previously stated that samples of prolactin and F.S.H. received from Doctor Riddle (Campbell, 1936) did not increase the liver fat of rats in doses of 20 mgm. and 5 mgm. per rat respectively. Later work demonstrated (Best and Campbell, 1938) that rats were relatively resistant to this effect and that if larger doses had been given the F.S.H. fraction would probably have increased liver fat in this species.

We were surprised to find that the liver fat activity did not parallel ketogenic activity, since there is much physiological evidence to support the view that, under certain conditions, the processes governing ketogenesis and increase in liver fat are linked together (Geelmuyden, 1923; Leathes and Raper, 1925). The results of the fractionations, however, indicate that these two effects are not due to the same substance. This is supported by the finding that L.F.A. is most stable to heat at pH 3.5 while ketogenic activity is most stable about pH 8.0. In addition, the ketogenic activity is more rapidly destroyed by heat than the L.F.A.

The high ketogenic activity of the globulin fraction appeared to be paralleled by high diabetogenic activity, so that these activities may be associated. Both are readily inactivated by heat. In this connection Shipley and Long (1938) observed that growth, glycosuric and ketogenic activities accompanied one another during the fractionation of anterior pituitary extracts. At the present time we do not know of any extract possessing higher diabetogenic activity than the "globulin" fraction.

In other laboratories (Young, 1938; Shipley and Long, 1938; Houssay and Biasotti, 1938) it has been found that prolactin is not diabetogenic. This is supported by our results, since large doses of prolactin administered to normal dogs did not produce signs of diabetes. The prolactin preparations were low in ketogenic and high in crop gland stimulating activity. This is in agreement with the previous observations of Shipley and Long (1938) who concluded that prolactin was not "of chief importance" in the production of ketogenesis by anterior pituitary extracts.

SUMMARY

1. From fractionation of anterior pituitary extracts it has been found that liver fat activity is not necessarily associated with prolactin, ketogenic or melanophore-expanding activity. Prolactin is not associated with ketogenic or diabetogenic activity, but the latter two appear to run parallel during fractionation.

2. Liver fat activity is rapidly destroyed by pepsin and trypsin.

3. Liver fat activity is most stable to heat at pH 3.5.

4. Ketogenic activity is most stable to heat at pH 8.0 and is more readily destroyed than liver fat activity.

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THE INFLUENCE OF ADRENALECTOMY AND OF FASTING ON THE INTESTINAL ABSORPTION OF CARBOHYDRATES

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An integral part of the picture of adrenal insufficiency, as Verzar describes it (1936, 1939), is the failure of selective intestinal absorption of metabolizable sugars. This assertion rests upon studies made by Wilbrandt and Lengyel (1933) in Verzar's laboratory on the absorption of glucose and of a nonmetabolizable sugar, xylose, in adrenalectomized rats. They found, in 13 adrenalectomized rats, that 41 per cent of the glucose placed in intestinal loops was absorbed in 1 hour as compared to 71.6 per cent absorbed by normal animals, whereas, in the case of xylose, they found no difference between the normal and the adrenalectomized rats. Further work from Verzar's laboratory claims the same to be true for cats (Issekutz, Laszt and Verzar, 1938) and for frogs (Minibeck, 1939). Cori had also previously (1927) reported a decrease in absorption of glucose in adrenalectomized rats. Deuel, Hallman, Murray and Samuels (1937) do not find a decrease. Althausen, Anderson and Stockholm (1939) agree with Deuel et al. only if a salt supplement is added to the diet; but if, on the other hand, the salt supplement is withheld, they confirm Verzar.

We were impressed, as have been others (Verzar, 1936; Marrian, 1937), by the significance it would have if it were to be established that an underlying difficulty in adrenal insufficiency is the failure of phosphorylation of glucose by the mucosa of the intestine resulting in a decrease in absorption rate to that of purely nonselective physical processes. The osmotically active glucose might, under these circumstances, cause a shift of water and electrolytes from the body fluids and tissues into the intestinal lumen, such as occurs into the peritoneal cavity following an intraperitoneal injection of glucose (Schechter et al., 1933; Gilman, 1934; Darrow and Yannet, 1935). The diarrhea which occurs in many adrenalectomized animals could also be explained in this way.

We therefore undertook to study again the intestinal absorption of sugars in adrenalectomized rats. I wish to express my thanks to Prof. Robert Gaunt for his interest and help in this work.

Methods. In testing Verzar's contention it seemed desirable not to alter the technique adopted in his laboratory which utilizes intestinal loops, despite the objections inherent in the method. These are principally the use of anesthesia and the trauma involved in laparotomy and manipulation of the intestine. Cori's technique (1925) of administering the glucose by stomach tube to unanesthetized animals avoids these objections but introduces new difficulties, such as the influence of variations in gastric and intestinal motility and emptying time on the absorption from the gut.

Absorption was studied in adrenalectomized, in sham operated, in non-operated fasted and in non-operated non-fasted rats. Male rats weighing 200 to 300 grams were used. Stock diet was allowed ad lib and a record of the daily intake was kept. Adrenalectomies were performed three days before the experiment and the rats immediately given access to 0.9 per cent saline as drinking fluid. This was continued until absorption was determined. Some of the controls, likewise, were given saline for three days before determining absorption. Others received tap water. When adrenalectomized animals received only tap water to drink during this three day period, they were unable to withstand the stress of the subsequent experimental procedure. The adrenalectomized and control animals were fasted for the 48 hours before absorption was determined.

The animals were anesthetized with intraperitoneal injections of 0.05 cc. of nembutal (6.5 per cent solution) per 100 grams' body weight. The adrenalectomized animals were more sensitive to the nembutal and required only $\frac{2}{3}$ of this dose. The sham-operated rats required the full dose. The abdomen was opened by midline incision and a loop of gut was measured off starting at the beginning of the jejunum. Measurements were accurately checked after the death of the animal. The absorption was calculated as milligrams per inch of gut.

The loop was cannulated at both ends and gently washed with warm 0.9 per cent saline. The abdomen was closed with clamps and the animal allowed to rest for one hour. The animal was kept warm during the entire experiment by means of hot water bottles. At the end of the hour, 3 cc. of isotonic sugar solution were introduced into the loop and the rubber tubing at the ends of the cannulae clamped so that there could be no leakage. After exactly $\frac{1}{2}$ hour the unabsorbed solution was removed and the gut washed three times with 3 cc. portions of normal saline. These washings were added to the recovered solution and the whole analyzed in duplicate for sugar by the Hagedorn-Jensen method.

In order to aid in analysis of our results the following further controls were used. Rats were subjected to sham operation. This consisted of exposing the adrenals by bilateral lumbar incisions. In some cases the adrenals were also gently freed from the kidney and the surrounding fat was removed. In others the adrenals were exposed but not disturbed.

Twenty-four hours later the food was removed, and the animals fasted for 48 hours before absorption of glucose was determined. Some of this group were given saline and some, tap water as drinking fluid. Other rats which underwent the same operative procedure were allowed to survive and were followed for over two months during which time they showed no signs of adrenal insufficiency.

We also compared the absorption of glucose and of xylose in normal animals, which had been allowed the usual stock diet up until 2 to 4 hours before the experiment, with that in normal rats which had been fasted for 48 hours. In addition, 4 rats were allowed 7 grams of food each (i.e., the

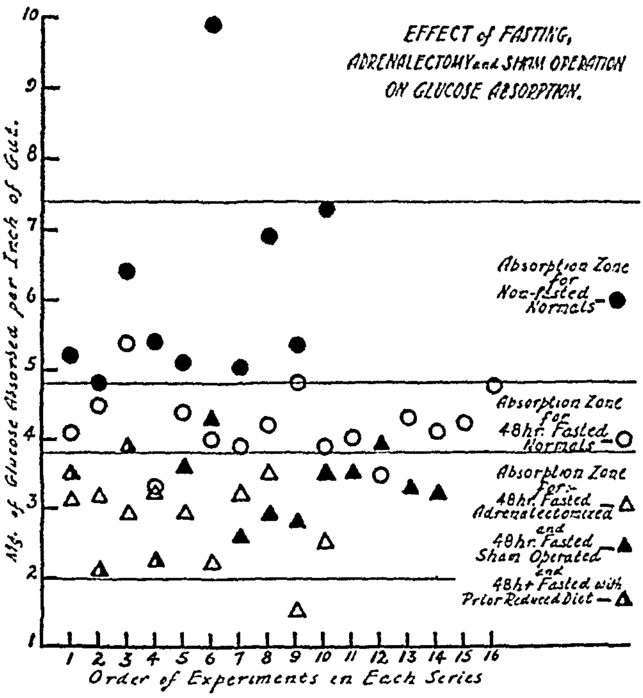

Fig. 1

average amount taken by the adrenalectomized or sham-operated rats during the 24 hour period following operation) for a period of 24 hours. Following this the food was removed entirely and the rats fasted for 48 hours. This made their food intake equal and comparable to that of the sham-operated and adrenalectomized rats for the 72 hour period preceding the experiment. Then absorption was determined as in the other rats.

Results. The results divide themselves into two classes. One consists of the values for the absorption of glucose, which vary with the conditions set; and the other, of the values for absorption of the non-metabolizable sugar, xylose, which remain fixed under the various experimental conditions.

The scatter diagram in figure 1 includes all the results on the absorption

of glucose. They are seen to fall into three distinct zones with only a small degree of overlap. The zone of maximum absorption is occupied by the values for the non-fasted normal animals. An intermediate zone is constituted by the values for 48-hour fasted normal animals, while in the zone of minimum absorption are fairly evenly interspersed the values for both adrenalectomized and sham operated 48-hour fasted animals. Also in this group are found the values on four animals which, previous to a 48 hour fast, had had their food intake restricted to the level to which that of adrenalectomized and sham-operated animals drops voluntarily following operation.

The lack of any corresponding effect of adrenalectomy on xylose absorption is illustrated in figure 2. The lack of influence of fasting on xylose absorption is shown in figure 3. These rats are a later series than those in figure 2, and show a higher absorption rate, but there is no difference between the non-fasted and fasted rats studied at the same time within the

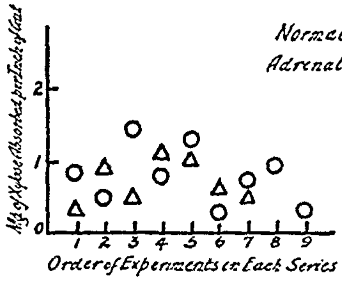

Fig. 2. Non-effect of adrenalectomy on xylose absorption

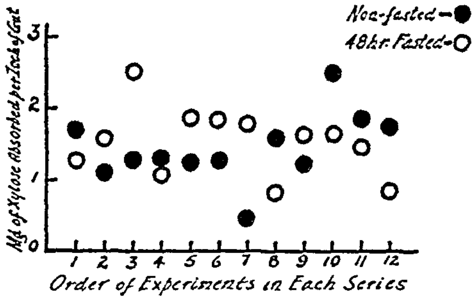

Fig. 3. Non-effect of fasting on xylose absorption

series. Variations in different series have also been observed for fructose absorption (Cori and Cori, 1928).

In no group is there any difference between those rats receiving tap water and those receiving saline as drinking fluid in regard to absorption of either glucose or xylose. (This does not include the adrenalectomized rats since they could not be maintained on tap water.)

Among the sham-operated rats there was no difference in absorption between those animals in which the adrenals had been merely exposed, and those in which these glands had been freed from the kidney and perirenal fat.

The normal average daily food intake was 13.5 grams. This dropped to an average of 7 grams the day following adrenalectomy or sham operation.

Thus adrenalectomy, sham operation or simple fasting reduces the absorption of glucose from the jejunum but does not affect that of xylose.

Discussion. The results of these experiments agree with the findings from Verzar's laboratory (Wilbrandt and Lengyel, 1933) in that the absorp-

tion of glucose is diminished after adrenalectomy, though to a smaller extent than they report, while the absorption of xylose is not affected. Our experiments show too, however, that fasting also limits subsequent absorption of the preferentially absorbed sugar, glucose, but not of xylose. In fact, it seems that fasting could account for the effect of adrenalectomy on the absorption of carbohydrates. We have shown that on the day following operation, adrenalectomized and sham-operated animals eat on the average about 7 grams of food instead of their usual average of 13.5 grams. This suggests that it is the post-operative anorexia that accounts for the decrease in absorption to below that of 48 hour fasted non-operated rats, by prefixing a period of subnormal intake, and thus in effect increasing the total period of fast. This is supported by the same low level of absorption obtained in animals in which the same period of subnormal intake was enforced preceding the 48-hour fast, but which were not subjected to either adrenalectomy or sham operation.

The findings on sham-operated animals appear to be at variance with those of Wilbrandt and Lengyel but, since the length of the post-operative interval and the regime on which the rats were maintained are not given, it is difficult to interpret their experiments.

The results of Deuel et al. (1937), who studied absorption by the Cori technique, in rats 12 to 20 days post-operatively, may not be strictly comparable to those reported here because of the long post-operative period intervening in their experiments, whereas ours were obtained three days post-operatively to conform to the experiments of Wilbrandt and Lengyel. Nevertheless in Deuel's experiments the effect of fasting on absorption does not seem to have been sufficiently taken into account. Judging from our results, as well as from those of Cori (1927), the extra 32-hours fast to which the controls were subjected might have lowered the absorption rate to that of the adrenalectomized rats which were fasted only 16 hours. Interpreted in this way their findings are not in disagreement with those reported here.

The present results disagree with the view of Althausen et al. (1939) that sodium chloride administration removes the difference in glucose absorption between adrenalectomized and control animals, since in our experiments the difference was obtained despite the sodium chloride supplement. Althausen's adrenalectomized animals had access to sucrose for the first 8 hours of the "fast" period, the amount taken being determined presumably by their appetite. The fasting period in these adrenalectomized animals is therefore obviously less than that in the controls, and this would modify the results as pointed out in the discussion of Deuel's work. Accordingly, had the sodium chloride restored the absorption to normal, then those animals the fast of which was shortened by sucrose, though adrenalectomized, should have shown a higher absorption rate than the controls. The

effectiveness of the carbohydrate fraction of the previous diet in influencing carbohydrate absorption has been shown by Westenbrink (1934, 1936). In explaining the apparent effect of sodium chloride, it seems likely that this was indirect—i.e., the well known one of diminishing anorexia. Consequently, the rats on sodium-chloride supplement probably ate more food through the entire post-adrenalectomy period and were able, as well, to take better advantage of the sucrose allowed for the first 8 hours of the "fast". No figures are given for the amounts of the food and sucrose intake so that the extent of these effects cannot be accurately gauged.

The difference between the results of the last two groups of investigators and those reported here cannot be attributed to differences between the Cori technique and ours, since Cori has also reported a diminution in absorption in adrenalectomized rats (1927).

The definite decrease in absorption of glucose, though not of xylose, in adrenalectomized animals, even when supported by saline, closely resembles the decrease in control animals the food intake of which has fallen or been reduced to that of the adrenalectomized animals. This suggests that the decreased absorption may be attributed as convincingly to the effects of anorexia as to a primary disturbance in the mechanism of absorption.

SUMMARY AND CONCLUSIONS

1. The absorption of glucose, a metabolizable sugar, and of xylose, a non-metabolizable sugar was studied in adrenalectomized, in sham-operated, in non-operated fasted, and in non-operated non-fasted rats.
2. The absorption of glucose in adrenalectomized rats even though on a sodium chloride supplement was decreased, whereas that of xylose was not.
3. Sham-operated rats showed a like decrease in the absorption of glucose.
4. The food intake of both adrenalectomized and sham-operated rats was found to decrease post-operatively.
5. Restriction of food intake or fasting was found in itself to decrease absorption of glucose but not of xylose in non-operated animals.
6. It appears that the reduction of food intake, induced by post-operative anorexia, and the surgical manipulation can account for the decrease in the absorption of glucose in adrenalectomized animals. It is probable, in fact, that anorexia alone can account for it.

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CONTROL OF CORONARY BLOOD FLOW IN THE HEART-LUNG PREPARATION

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Recently Wiggers (1) has discussed the currently accepted views on the principal factors affecting coronary circulation. Changes in aortic pressure, the vasomotor mechanism, the action of metabolites and mechanical influences such as the massaging action of the myocardium on the cardiac vessels, are considered by different investigators as the chief mechanisms responsible for the regulation of coronary blood flow.

The desirability of further work on the control of coronary blood flow in the heart-lung preparation was indicated by a few preliminary experiments done in this laboratory. Since some of the results obtained were not in complete accord with the literature it seemed advisable to reinvestigate certain phases of the problem with a method of measuring blood flow not previously used in experiments of this nature.

We are reporting here the results of a study on the influence of 1, changes in the mean arterial blood pressure; 2, changes in cardiac output; 3, stimulation of the cardiac branches of the stellate ganglion, and 4, changes in the heart rate on the coronary blood flow of the heart-lung preparation of the dog.

METHODS. Previous investigators working with the heart-lung preparation measured coronary sinus outflow by means of a Morawitz-Zahn cannula or determined coronary inflow by the use of the hot wire anemometer, which was connected to a cannulated perfused coronary artery. In the present experiments coronary inflow was measured with the thermostromuhr which caused a minimal disturbance of the heart and its vessels (2, 3). The heart-lung preparation was made under pentobarbital sodium (nembutal) and the thermostromuhr unit was applied to the circumflex branch of the left coronary artery either before or after the heart-lung preparation was completed. Application of the unit before preparation of the heart and lungs facilitated obtaining hemostasis. Twenty-three heart-lung preparations were made. The operative pro-

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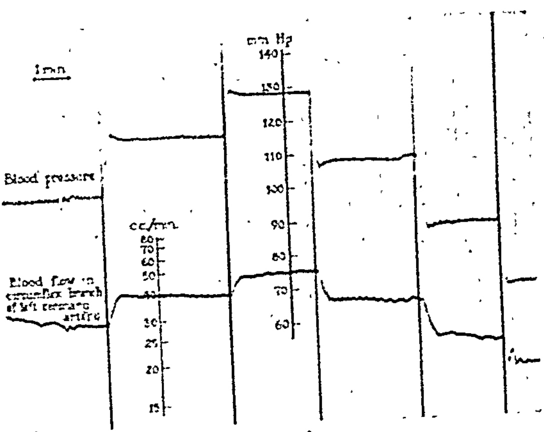


Fig. 1

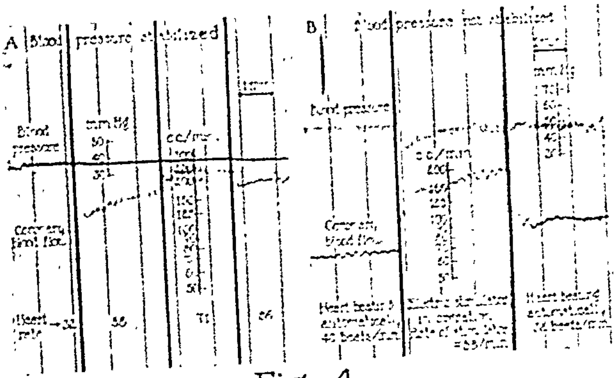


Fig. 4

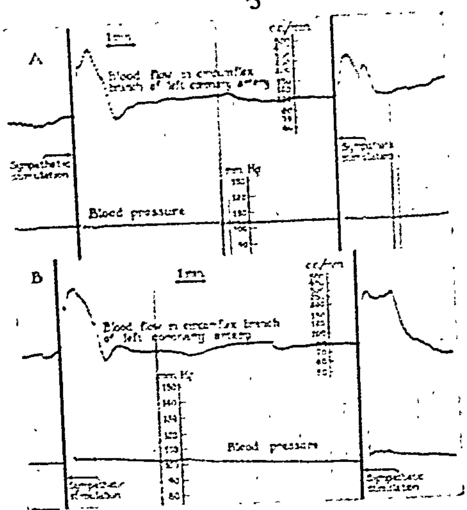
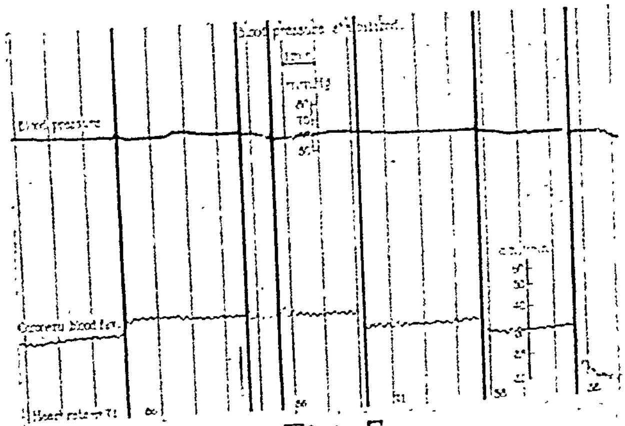


Fig. 2



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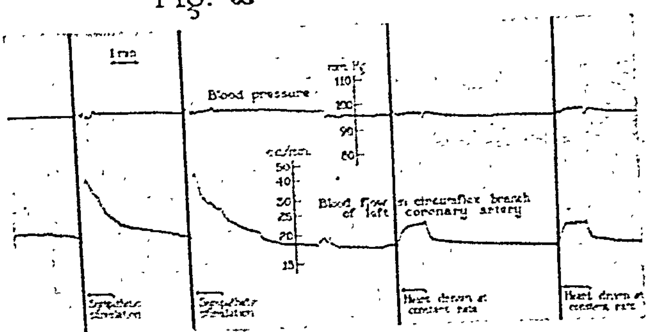

Fig. 3

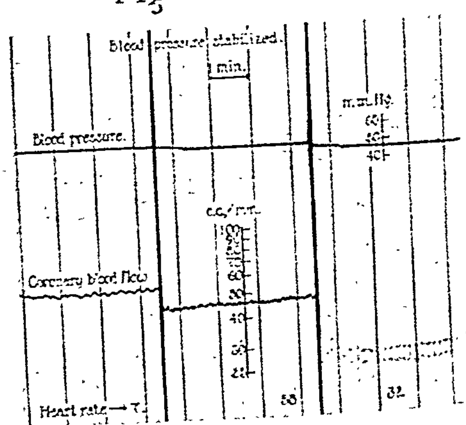

Fig. 6

Figs. 1–6

Fig. 1. Photographic record showing the effect of changes of blood pressure on the coronary blood flow of the heart-lung preparation. The blood flow in all the experiments was measured in the circumflex branch of the left coronary artery.

Fig. 2. Upper tracing, photographic record of coronary blood flow. Lower tracing, record of blood pressure. At the point indicated by the heavy vertical lines the cardiac branches of the stellate ganglion were stimulated (inductorium). Blood pressure in A was kept constant but about fifteen seconds were required for equilibrium of blood pressure in B.

cedures which usually lasted one and a half hours included setting up the heart-lung, applying the thermostromuhr unit to the coronary artery and the preparation of the sympathetic nerves for stimulation on one or both sides. The sympathetic nerves were prepared for stimulation by sectioning all of the branches of the stellate ganglion except the cardiac branches (ansa Vieusseni). Cardiac output was measured directly; the blood pressure and coronary flow were optically recorded. The arterial blood pressure was controlled by a method similar to that described by Greene (4).

Blood pressure and coronary flow. In analyses of the dynamics of coronary circulation in the denervated heart-lung preparation, arterial blood pressure has repeatedly been demonstrated to be the chief factor influencing coronary blood flow (5–10). In repeated experiments we have likewise found that an increase in the mean arterial pressure of the heart-lung preparation caused an augmented coronary flow and a decrease of blood pressure resulted in a diminished coronary flow (fig. 1). By use of another method of measuring coronary flow mean arterial pressure has again been shown to be an important and determining factor in the control of coronary flow in the denervated heart-lung preparation as the data of a typical experiment indicate (table 1).

Cardiac output and coronary flow. By keeping the blood pressure and temperature of the perfusing blood constant, the uncomplicated effect of changes in cardiac output on coronary flow was studied. The venous inflow into the heart was increased or decreased at will by raising or lowering the blood reservoir. In a compensating heart an increased output resulted from the former.

Our results confirm the observations of previous workers (5, 11, 12). Augmenting cardiac output as much as 275 per cent above the basal level did not increase coronary flow more than 3 to 6 per cent. This increase, however, is well within the limits of the error of the method and cannot

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Fig. 3. Upper tracing, photographic record of blood pressure. Lower tracing, photographic record of coronary blood flow. The first and second heavy vertical lines indicate the points at which the cardiac branches of the stellate ganglion were stimulated. At the third and fourth heavy vertical lines the heart was accelerated from 164 to 212 beats per minute. The blood pressure was controlled.

Fig. 4A. The effect on coronary blood flow of driving the heart at successive increased rates, the blood pressure being kept constant; B, the effect of imposing an increased rate on the automatically beating heart with the blood pressure uncontrolled.

Fig. 5. An increment in the imposed rate of the heart above 86 beats per minute did not increase the coronary blood flow but each successive decrement in heart rate reduced the coronary blood flow.

Fig. 6. Decrements in the imposed rate of the heart resulted in significant decrements in coronary flow.

be explained in our experiments by changes in mean arterial blood pressure since the latter was constant. Even sudden marked changes of cardiac output in the denervated heart did not influence the coronary flow, in contrast to the effect which increase of cardiac output is reported to have on coronary flow in the innervated heart. According to Anrep, the increase in coronary flow with increased cardiac output in the innervated heart is of reflex origin and disappears after section of the vagi.

Influence of vasomotor nerves on coronary flow. It is evident from the work of many that the coronary circulation in the intact animal is affected by factors in addition to the influence of the mean arterial blood pressure. According to numerous researches nervous influences play a prominent rôle in this regard. Wiggers recently reviewed the subject critically and discussed the difference in opinion of various investigators concerning the

TABLE 1

Relation of changes in coronary flow and cardiac output to changes in blood pressure

	BLOOD PRESSURE		CORONARY FLOW		CARDIAC OUTPUT	
	Mm. Hg	Per cent increase	Cc. per minute	Per cent increase	Cc. per minute	Per cent increase
Control values	70		25		132	
Values after blood pressure was increased 30 mm. Hg	100	+43	33	+32	220	+67
Values after blood pressure was increased 45 mm. Hg	115	+64	41	+64	194	+47

effect of sympathetic and parasympathetic stimulation on the coronary vessels.

Sympathetic stimulation increases markedly the force of left ventricular contraction and raises the arterial pressure. Increased arterial pressure alone augments coronary flow and the augmentation of coronary flow on sympathetic stimulation could be attributed to a summation of both factors. Wiggers has stressed the importance of changes in blood pressure and heart rate resulting from vasomotor stimulation. The early workers assumed that the peripheral resistance of the heart-lung apparatus represented the blood pressure of the preparation but when the dynamics of the heart are rapidly changing as with sympathetic stimulation, this assumption is erroneous. In order to study the effect of the vasomotor action of the sympathetic nerves, the mean arterial pressure must be controlled. To accomplish this in our experiments a rubber tubing was joined to the arterial side of the heart-lung preparation and connected with a flask, which could be raised to a height of about 2 meters. The surplus of blood ejected during the increase in force of the ventricular contraction

was carried into this flask. The mean arterial blood pressure was effectively equalized by this method (4). The blood pressure usually remained on a stabilized level during sympathetic stimulation (fig. 2A). Occasionally at the beginning of stimulation a few seconds were required for the blood pressure to come to equilibrium (fig. 2B).

In the present series of experiments the cardiac branches of the stellate ganglion of one side or the other were stimulated by means of an inductorium. The strength of the current was varied by changing the position of the induction coil from 12 to 4 cm. Sympathetic stimulation was usually carried out in the beginning because sometimes the heart failed to respond to the stimulus later in the experiment. The sympathetic nerves on one side were usually stimulated for thirty seconds. Coronary flow

TABLE 2

Effect of sympathetic stimulation on coronary flow and cardiac output under uncontrolled blood pressure

	HEART RATE		UNCONTROLLED BLOOD PRESSURE		CORONARY FLOW		CARDIAC OUTPUT	
	Beats per minute	Per cent increase	Mm. Hg	Per cent increase	Cc. per minute	Per cent increase	Cc. per minute	Per cent increase
Control values	138		76		57		92	
Values resulting from sympathetic stimulation for thirty seconds	150	+8.7	82	+7.9	300	+426	268	+191
Control values	150		75		25.5		226	
Values resulting from sympathetic stimulation for thirty seconds	174	+16	104	+39	52.5	+106	592	+162

before and after stimulation was continuously photographed and the heart rate and cardiac output were determined. In every instance stimulation of the sympathetic nerves was followed by a large augmentation of coronary flow. In none of our experiments was a decrease in coronary flow observed, regardless of the strength of stimulus. Increases in coronary flow on stimulation of the cardiac branches of the stellate ganglion varied from values of 28 to 426.0 per cent. On the average, the increase was about 150 per cent. The percentage increase in flow on stimulation of the sympathetic nerves reached similar high values when mean arterial blood pressure was kept constant. Data on changes in coronary flow with and without blood pressure control are presented in tables 2 and 3.

Morawitz and Zahn (8), in their experiments, noted that the coronary outflow on sympathetic stimulation showed a much greater increase than could be explained by the accompanying increase in blood pressure.

We are well aware of Wiggers' findings that mean arterial blood pressure can remain constant and that significant changes in pulse pressure may alter coronary flow. In our experiments, the cannulated innominate artery carried the blood from the heart and the rubber tubing of the equalizer was connected distal to this branch of the aorta. Mean blood pressure was recorded optically from a needle inserted in the tubing near the arch of the aorta. The increase in magnitude of ventricular contraction and the changes in systolic and diastolic arterial pressure, mean pressure remaining unaltered, possibly may have been an additional factor in the augmentation of coronary flow, but we question whether increases of more than 400 per cent were owing to possible changes in pulse pressure alone. As shown in the next section of this paper, increases in heart rate produced by electrical means are capable of causing a significant

TABLE 3

Effect of sympathetic stimulation on coronary flow and cardiac output under controlled blood pressure

	CONTROLLED BLOOD PRESSURE	CORONARY FLOW		CARDIAC OUTPUT	
		Cc. per minute	Per cent increase	Cc. per minute	Per cent increase
	mm. Hg				
Control values	104	100		314	
Values resulting from sympathetic stimulation for thirty seconds	104	350	+250	380	+21.0
Control values	103	123		268	
Values resulting from sympathetic stimulation for thirty seconds	103	350	+184.6	282	+5.2

augmentation in coronary blood flow. However, when all the known factors are considered it is difficult to account adequately for the results of sympathetic stimulation in the heart-lung preparation without invoking vasodilatation of the coronary vessels.

Influence of heart rate on coronary flow. By changing the temperature of the perfusing blood, by cooling or warming the sinal node, or by stimulating the sino-auricular node by an electrical stimulator the heart rate can easily be changed.

Porter (7) and Langendorff (6) explained increases in coronary flow due to increases in rate on the basis of the massaging action of the heart. Morawitz and Zahn (8) accelerated the heart beat by warming the sinal node and observed a 50 per cent decrease in flow from the coronary sinus. Cooling the sinal node decreased arterial pressure and coronary flow. The decrease in coronary flow during acceleration of the heart rate could

not be entirely explained, in the opinion of these writers, by the fall in blood pressure. Nakagawa (13) noted, in his experiments, that a decrease in temperature from 38°C. to 31°C. was accompanied by a decrease in heart rate from 192 to 120 beats per minute and, simultaneously, coronary sinus output was increased from 27 cc. to 43 cc. per minute. In a second series of Nakagawa's experiments, the sino-auricular node was stimulated with single induction shocks. The venous inflow, the blood pressure, and the temperature of the perfusing medium were kept constant. In these experiments, in which heart rate was changed from 102 beats to 132 beats or from 156 to 186 beats per minute, coronary flow remained constant during stimulation. Hammouda and Kinosita (14) confirmed, in experiments on the isolated heart, the observations in the heart-lung preparation—that the coronary flow, even within wide ranges of heart rates, is not altered, and only excessive acceleration leads to decreased flow.

Miller, Smith and Graber (15) studied the influence of acceleration of the heart beat on coronary flow in the isolated heart and the heart in situ. The isolated heart of a rabbit was stimulated with rhythmically induced shocks at different rates. Blood pressure and temperature were kept constant. The coronary sinus flow was measured with a Morawitz-Zahn cannula. Acceleration of cardiac rate from 120 to 176 beats per minute increased coronary flow about 28 per cent. Acceleration in rate from 176 to 196 beats per minute augmented coronary flow about 4.5 per cent. While acceleration of the heart beat always was followed by increased coronary flow, a greater increase was obtained if the initial cardiac rate was approximately 120 beats per minute and the heart accelerated at least 50 beats per minute. The increase in coronary flow during acceleration was less pronounced if the heart rate, before the stimulation, was about 150 beats per minute.

In a second series of experiments on the intact heart the authors just named applied small thin rubber bags to the sino-auricular node. Changes from cold to warm increased heart rate and coronary flow.

Anrep and Häusler (16) studied the effect of increased heart rate on coronary flow with the hot wire method. The rate of ventricular contraction was at first reduced by cooling the sino-auricular node or cooling the whole heart. The ventricle then was stimulated with single induction shocks and the heart accelerated from the initial rate to rates at which it did not follow all stimuli with regular contractions. The effect of acceleration is considered by these authors to depend on the change of duration and strength of premature ventricular contractions, and the coronary flow is determined by the sum of the two factors. The result may lead to an augmentation or diminution of coronary flow. In later experiments, Anrep (17) did not find any significant change during acceleration of the heart in the heart-lung preparation.

Hochrein, Keller and Mancke (18), studying coronary flow with Anrep's hot wire method and Broemser's tachograph, found that warming the sino-auricular node often increased coronary flow to values as high as 100 per cent even if the heart rate was not accelerated.

In the first series of our experiments, the heart rate was changed from the automatic rate to a different frequency by means of the electric stimulator of Hill (19). One electrode was placed near the sino-auricular node in the right auricle; the second electrode was applied near the root of the aorta. The heart was stimulated for thirty seconds and only occasionally for one minute. Heart rate and cardiac output were determined and coronary flow and the uncontrolled blood pressure were photographed throughout the experiment.

TABLE 4

Effect of acceleration of heart rate on coronary flow and cardiac output

	Uncontrolled blood pressure		Heart rate		Coronary flow		Cardiac output	
	Mm. Hg	Per cent increase	Beats per minute	Per cent increase	Cc. per minute	Per cent increase	Cc. per minute	Per cent decrease
Control values	100		124		58		344	
During stimulation with interrupter	100	0	148	+19.4	76	+31	248	−28
Control values	101		132		60		328	
Stimulation with interrupter	101	0	164	+24.2	82	+36.7	256	−22
Control values	93		176		52		208	
Stimulation with interrupter	97	+4.3	200	+13.6	77	+48.1	152	−27

In thirty-six observations on the heart-lung preparation, in which an accelerated rate was imposed on the heart, the coronary flow showed a marked and definite augmentation. Typical examples are given in table 4.

Usually the coronary flow increased from 30 to 50 per cent during the time of stimulation. Occasionally the increase in coronary flow was as high as 150 per cent, but never reached the average values which were obtained on stimulation of the sympathetic nerves. The coronary flow increased only during the time of the stimulation and returned to near the previous level as soon as the heart rate returned to the control value (figs. 3 and 4B).

In a few of our experiments a decrease instead of an augmentation of coronary flow was observed during the period of acceleration. In almost all of such experiments, a drop in blood pressure was noted. However, in some of these experiments, in spite of a decrease in blood pressure, the coronary flow was augmented. Many times considerable difficulty

was experienced in getting the heart to follow the rate of stimulation. Such experiments were considered failures and were not included in this report.

The response in coronary flow to acceleration of heart rate could be elicited throughout the course of the experiments. Whether the heart was driven early or late in an experiment was of importance only because coronary flow progressively increased with the duration of the heart-lung preparation. This has been a common observation of all of those who have worked with this preparation.

The experiments just described show that the coronary flow is increased when the rate of the automatically beating heart is accelerated by means of Hill's stimulator. It seemed important to determine whether changes in the induced rate of the heart would cause corresponding changes in the coronary blood flow. Therefore, another series of observations was made in which the heart rate was controlled throughout the experiment. The effect on coronary flow of increasing and decreasing the heart rate was observed while all other factors were kept constant. As a result of these observations it can be stated that within limits which vary with different preparations, increases in heart rate cause significant increments and decreases in heart rate result in definite decrements in coronary blood flow as will be indicated by the following data.

In the experiment now being described the heart was caused to beat 32 times per minute and the coronary flow was 70 cc. per minute. The rate of the heart was increased to 58 beats per minute which caused an increase in coronary flow to more than 150 cc. per minute. An increase in rate to 71 beats per minute resulted in the coronary flow reaching nearly 250 cc. per minute. However, when the apparatus was adjusted to drive the heart at 86 beats per minute the coronary flow was not augmented but slightly decreased (fig. 4A). In another series of observations the initial induced rate was 71 per minute and the coronary flow was about 35 cc. per minute. On increasing the rate to 86 the coronary blood flow increased to about 40 cc. per minute but an additional increment in the rate of stimulation of the heart did not increase the coronary blood flow since the heart did not beat at the increased rate. Successive decrements in heart rate of 15, 28 and 54 decreased the coronary flow from a control of, 50 cc. to less than 20 cc. per minute (fig. 5). In another series of observations the heart was driven at 71, 58 and 32 beats per minute and the resulting coronary blood flow was approximately 54, 46 and 28 cc. per minute respectively (fig. 6).

In one experiment, the temperature of the perfusing blood was increased from 35.5°C. to 41°C. The heart rate accelerated from 120 to 154 beats per minute. The mean arterial pressure was kept constant. Coronary flow increased from 40 cc. to 67 cc. per minute.

The augmentation of coronary flow during acceleration of the heart rate cannot be easily explained. The controlled blood pressure during stimulation remained relatively constant. The coronary flow was sometimes increased while the uncontrolled blood pressure was decreased during the acceleration of the heart. Therefore, our results cannot be accounted for on the basis of changes of blood pressure.

The possible rôle of metabolites has been considered. We have shown that increasing the cardiac output as much as 275 per cent did not appreciably alter the coronary flow. Since blood pressure was kept constant the work of the heart was proportionately increased when the cardiac output was augmented. Under these conditions an increase in metabolites sufficient to affect the coronary vessels did not apparently occur. This being true one hesitates to advance the hypothesis that metabolites were responsible for the increased coronary flow accompanying the acceleration of the heart rate in view of the fact that the cardiac output was reduced and therefore the work of the heart was decreased in nearly all of the experiments in which the heart was driven at an increased rate.

The increase in coronary flow might conceivably have resulted from incidental stimulation of the cardiac nerves with a consequent vasodilatation of the coronary vessels. Attempts to eliminate the possible influence of the sympathetic nerves by injections of ergotoxine failed, as successive large doses of this drug did not prevent stimulation of the cardiac branches of the stellate ganglion from exerting its usual effect.

As already stated, stimulation of the sympathetic nerves was very effective in augmenting coronary flow when done early but ineffective when done late in an experiment. Since induced acceleration of the heart rate caused increases in coronary flow without respect to the life of the heart-lung preparation, the likelihood is not great that the sympathetic nerves were responsible for the increased coronary blood flow resulting from driving the heart at induced accelerated rates.

The presence of chemically active substances resulting from stimulation of the heart cannot be ignored as a possible factor in the increased coronary flow. An increased massaging action of the heart muscle during acceleration as stressed by Porter and Langendorff might be suggested as a factor in producing the augmented coronary flow but according to Wiggers this influence is of minor importance. That a decrease in the effective peripheral resistance of the coronary vessels occurred cannot be doubted but the factor or factors responsible for it are not apparent.

SUMMARY

In the heart-lung preparation the influence of various factors on coronary flow was studied with the thermostromuhr. In agreement with others an intimate dependence of coronary flow on mean arterial blood

pressure was found. Changes in cardiac output had no effect on coronary flow, if the arterial pressure and the temperature of the perfusing medium were kept constant. Stimulation of the cardiac branches of the stellate ganglion of the heart-lung preparation augmented coronary flow on an average of about 150 per cent. Similar values for coronary flow were obtained in the presence of a constant arterial blood pressure. The increase in coronary flow resulting from stimulation of the sympathetic nerves of the heart-lung preparation is thought to be due at least in part to a vasodilator action of the sympathetic nerves. An augmentation of coronary flow was demonstrated with acceleration of the heart rate by means of an electric stimulator. The augmented coronary flow was not due to an elevated mean blood pressure.

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THE CARDIAC OUTPUT IN REST AND WORK IN HUMID HEAT

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In humid, hot climates evaporation of sweat on the surface of the body is greatly impaired, and the flow of blood through the skin must be increased in order to maintain a normal body temperature. The effect of this changed distribution of the blood on the circulation and its regulation has great physiological interest and only few investigations in this field are available (e.g., Dill, Edwards, Bauer and Levenson, 1931; Scott, Bazett and Mackie, 1940). A new opportunity for studying this question came when the staff of the Harvard Fatigue Laboratory in the summer of 1939 went to Benoit, Mississippi, in order to make physiological studies under the climatic conditions present in that part of the world. When our data were being collected in Benoit, the mean temperatures were 30.6°C. dry bulb and 27.6°C. wet bulb. Supplementary data for comparison were collected mostly during the following winter in Boston.

The subjects were all members of the laboratory staff except for a Negro sharecropper (B. C.), studied in Mississippi, who came to Boston as a laboratory helper in October. E. A. reached Benoit directly from Denmark about ten days before the determinations were started, whereas the other subjects at the time of the determinations (July, 1939) had been in Benoit for about one and one-half months. The estimations of the cardiac output in rest and work were made by the Grollman acetylene method, which gives the arterio-venous O_2-difference; this divided into the metabolic rate gives the cardiac output per minute. The metabolisms were determined from the ventilation and analyses of the expired air. Experiments in rest and during work will be treated separately.

a. Rest. For our resting experiments the subjects came in fasting in the morning and were asked to lie down to a prone position (or to sit down with legs horizontal) until the pulse rate had become stationary (usually after about 30 min). At least two determinations of the cardiac output were made on each subject.

Table 1 (A) gives the results from Benoit. The values presented are averages of all the single determinations. Table 1 (B) gives corresponding data from experiments carried out in Boston during the following winter on four of the subjects, and further from determinations on E. A. and M. N.

during the preceding winter in Copenhagen, Denmark. These experiments were all made at ordinary room temperature (22°C.). No winter values were obtainable on S. R. It is quite evident from the data that the cardiac output per m². of body surface (the cardiac index of Grollman) is the same in Boston as in Benoit. The average value in Benoit is 2.44 l. per min. and per m²., in Boston, 2.49 (values for E. A. from Copenhagen not included). The individual changes are negligible except in the case

TABLE 1

Observations made in the resting state

	WEIGHT	SURFACE AREA	B.M.R.	A-V O_2 DIFF.	CARDIAC OUTPUT	CARD. OUTPUT / SURFACE AREA	PULSE	STROKE VOL.
(A) In Benoit								
	kgm.	*m².*	*cc. O_2/min.*	*cc./l.*	*l./min.*	*"card. index"*		*cc.*
E. A.	69	1.82	236	53	4.5	2.47	75	60
B. C.*	61	1.69	229	53	4.3	2.55	64	67
D. B. D.	76	1.95	225	51	4.4	2.26	56	79
M. N.	73	1.97	233	51	4.6	2.34	66	70
S. R.	61	1.70	220	52	4.2	2.47	74	57
J. W. W.	69	1.87	234	50	4.7	2.52	57	82
Average						2.44		
(B) In Boston and Copenhagen								
E. A.	70	1.83	249	46	5.4	2.95	57	95
B. C.*	67	1.78	254	56	4.5	2.53	55	82
D. B. D.	79	1.98	241	55	4.4	2.22	56	79
M. N.†	83	2.09	249	52	4.8	2.30	55	87
J. W. W.	70	1.88	235	50	4.7	2.50	60	79
Average						2.49		
E. A.†	70	1.83	254	48	5.3	2.90	59	90

* Colored.

† Copenhagen.

of E. A., who shows a decrease from 2.95 in Boston (2.90 in Copenhagen one year earlier) to 2.47 in Benoit. The pulse rates in Benoit were, in the cases here described, slightly higher than in Boston or in Copenhagen. Accordingly the stroke volume was lower in Benoit than in Boston.

There seemed to be a correlation between the external temperature at the moment of determination and the pulse rate, which is shown for the subject E. A. in table 2.

The practically unaltered cardiac index found in our subjects when ac-

climatized to hot, humid climate indicates that a regulation has taken place. In acute experiments, lasting only for one to two hours, a considerable shift of blood from the central vessels to the periphery usually is accompanied by a decreased cardiac output, pulse acceleration, and vasoconstriction, impairing the functions of organs as the intestine and the kidneys (Asmussen, Christensen and Nielsen, III, 1939). It seems likely that for short exposures to high temperatures similar changes will take place, but that a prolonged exposure must demand a different regulation, allowing all organs an adequate blood supply. Facts supporting this assertion are presented by Scott, Bazett and Mackie, 1940, who found that the cardiac output in their subjects was slightly increased on the first few days of exposure to heat; the original values were then regained. At the same time an increase in blood volume had taken place. Our subjects had all been exposed to the heat for at least ten days and therefore are comparable to the subjects of Scott, Bazett and Mackie in the later stages of acclimatization. It is, therefore, reasonable to assume, as these authors do, that

TABLE 2

Cardiac output and heart rate of subject E. A. at Benoit

SHADE TEMPERATURE	SUBJECT'S LOCATION	NUMBER OF DETERMINATIONS	PULSE RATE	CARDIAC OUTPUT
°C.				l./min.
29	Shade	1	70	4.6
30	Shade	2	74	4.5
32	Sun	2	81	4.5

the normal cardiac index found in fully acclimatized individuals is made possible by the increased blood volume. The subjects of Scott, Bazett and Mackie showed increases of as much as 25 per cent or more in blood volume during their stay in a hot room; of our six subjects, four had a slightly increased blood volume in Benoit, one (D. B. D.) showed no change, and one (E. A.) even showed a slight decrease. (See Forbes, Dill and Hall, 1940.) The fact that this subject alone had a lower cardiac index in Benoit than in cooler climates seems to indicate that an increased blood volume is an important factor in maintaining a normal cardiac output.

Small daily variations in the amount of blood pooled in the skin apparently were compensated for by changes in the pulse rate, as table 2 shows. A high pulse rate can, as pointed out by Asmussen, Christensen and Nielsen (I, 1939), compensate for a decreased filling of the great central veins.

Recapitulating, it seems reasonable to assert that after acclimatization to a hot, humid climate has taken place, the cardiac output in rest is the

same as in a more temperate climate, and that compensation for the increased skin circulation is accomplished by means of an increased blood volume and probably by a slightly raised pulse rate.

b. Work. The work consisted in walking on a treadmill at a speed of 5.6 km. per hr. at an elevation of 8.6 per cent. This grade of work could easily be maintained for two to three hours in Boston's temperate climate, whereas it was very difficult in Benoit and for some subjects impossible to continue the prescribed two hours. The difference can, perhaps, best be described by stating that whereas a "steady state" could easily be

TABLE 3

Observations made during first one-half hour of walk

	O_2-INTAKE	A-V O_2 DIFF.	CARDIAC OUTPUT	CARDIAC OUTPUT / BODY WEIGHT	PULSE	STROKE VOL.
			(A) In Benoit			
	l./min.	*cc./l.*	*l./min.*	*l./kgm.*		*cc.*
E. A.	1.60	108	14.8	0.21	141	105
B. C.*	1.47	117	12.6	0.21	132	96
D. B. D.	1.78	128	13.9	0.18	126	110
M. N.	1.79	120	14.9	0.20	146	102
S. R.	1.51	114	13.2	0.22	130	102
J. W. W.	1.81	111	16.3	0.24	137	119
Average		116	14.3	0.21		106
			(B) In Boston			
E. A.	1.86	101	18.4	0.26	120	153
B. C.*	1.63	113	14.4	0.22	140	103
D. B. D.	1.93	120	16.1	0.20	132	122
J. W. W.	1.63	117	13.9	0.20	120	106
Average		113	15.7	0.22		121

* Colored.

reached in Boston, the pulse rate and the body temperature of some subjects in Benoit were steadily increasing, eventually making continuance of work impossible. For comparison between the circulation rate at a given rate of work in Boston and in Benoit, we have chosen the first half-hour of work, where a relatively steady state is reached, before a failure of circulation or temperature regulation is evident, and the last half-hour of the work, where in Benoit signs of a circulatory failure were apparent.

Table 3 (A and B) shows the results for the first half-hour of work. It will be seen that the average arterio-venous O_2-difference for the whole group is practically the same in Boston as in Benoit. The small difference

in cardiac output is mainly due to the difference in oxygen intake during the work, which was lower in Benoit owing to the fact that all of these subjects lost weight during their stay there. A comparable expression for the cardiac output in this kind of work is the cardiac output per kilogram of body weight, and table 3 shows that this is practically unchanged. Although there are great individual differences, the pulse rate for the whole group is somewhat higher in Benoit than in Boston and the stroke volume somewhat lower.

TABLE 4

Observations made during last one-half hour of walk

	O_2 INTAKE	A-V O_2 DIFF.	CARDIAC OUTPUT	PULSE	STROKE VOL.	DURATION OF WORK
(A) In Benoit						
	l./min.	*cc./l.*	*l./min.*		*cc.*	*min.*
E. A.	1.82†	140	13.0	160	81	175
B. C.*	1.50	119	12.6	139	91	75
D. B. D.	1.78	131	13.6	138	99	75
M. N.	1.97	141	14.0	167	84	105
S. R.	1.56	111	14.1	162	87	120
J. W. W.	1.95	142	13.8	170	81	85
Average		131	13.5		85 (Δ = −19.8%)	
(B) In Boston						
E. A.	1.96	111	17.6	120	147	180
B. C.*	1.66	116	14.3	140	102	120
D. B. D.	2.04	128	15.9	140	114	120
J. W. W.	1.67	126	13.3	135	99	120
Average		120	15.3		116 (Δ = −4.1%)	

* Colored.
† Extrapolated.

As the work progresses, however, the influence of the hot climate is more marked. Table 4 (A and B) represents values obtained during the last half-hour of the work. The last column shows the time at which the work was interrupted, either deliberately or, in some cases, because of exhaustion. It will be seen that the arterio-venous O_2-difference, both in Boston and in Benoit, is higher at the end than at the start but that the more marked increase is in Benoit. The cardiac output is slightly decreased, more so in Benoit than in Boston. The greatest effect can be seen in the pulse rate and the stroke volume. Whereas the latter in Boston is only decreased by about 4 per cent, it is decreased by 20 per cent in Benoit.

The demands of the hot, humid climate on the cardiac output during work seem, according to our results, to have been practically compensated for in acclimatized subjects. There is good reason to believe that the compensation has been accomplished in the same way as in rest, i.e., by an increased blood volume and an increased pulse rate. In prolonged work this compensation seems to be incomplete. The steadily increasing body temperature in the less fit subjects indicates rising difficulties for heat dissipation. An increased skin circulation will impair the filling of the central veins and of the heart. A fall in cardiac output and blood pressure will call forth a reflected rise in the pulse rate. If this fails to maintain an adequate cardiac output, the blood pressure will drop to levels where continuance of work will be impossible. It is, however, not possible to say whether the high body temperatures reached in these experiments or the inadequate blood supply to muscles and brain are the direct cause of the ultimate exhaustion. That the organism has thrown in all its reserves of blood is made evident by the fact that some of the subjects vomited or suffered from water-diarrhea immediately after the work, indicating that the blood supply to the gastro-intestinal organs was too low to allow them to absorb water drunk during or after the work. (Compare Asmussen, Christensen and Nielsen, III, 1939.)

The above results are in agreement with the results of Dill, Edwards, Bauer and Levenson, 1931, who by acute exposure to high or low temperatures during work found the same effects on pulse rate and stroke volume as we have found and showed that the systolic blood pressure during work was lower in the hot room than in a cold one, indicating possibly a failing blood pressure regulation.

SUMMARY

Acclimatization to humid heat seems to involve such regulations that the circulation in rest and during work in a steady state can be kept at a practically normal level. A blood volume increased by about 5 per cent (see Forbes, Dill and Hall) and a slightly higher pulse rate are assumed to be the two main factors in this regulation. Circulatory failure during work develops rather fast in humid heat owing to the fact that the heat dissipation is made difficult. A larger amount of blood is demanded for the skin circulation, making maintenance of an adequate cardiac output increasingly difficult.

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THE RELATION OF FASTING EXTERNAL PANCREATIC SECRETION TO HUNGER[1]

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That a close relationship exists between the movements of the empty stomach and external pancreatic secretion was demonstrated by the extensive researches of W. N. Boldyreff (1), which amplified the reports of "periodic work" of the digestive canal by Russian investigators (Bruno, Schirokich, Tscheschkow, Klodnizki, Kaznelson, Edelman (2)). Boldyreff demonstrated that periods of motor activity of the empty stomach were accompanied by the secretion of intestinal juice, pancreatic juice and bile. These periods lasted 20 to 30 minutes and were followed by "rest" periods of $1\frac{1}{2}$ to $2\frac{1}{2}$ hours in which these organs were inactive, no motility or secretion being recorded. On this basis, Boldyreff postulated periodic "work" and "rest" for these organs in the fasting dog. Babkin (3) confirmed Boldyreff's conclusions but his experiments showed some pancreatic secretion during "rest" periods.

Since these early researches few confirmatory experiments have been conducted. E. B. Boldyreff (4) confirmed W. N. Boldyreff but concluded that concomitant gastric motility and pancreatic secretion were accompanied by a fall in blood-glucose of 20 to 30 mgm. per cent during the "work" periods. Zucker, Newburger and Berg (5) denied the existence of "periodic" pancreatic secretion, in animals having "total" drainage of the pancreatic juice by the method of Elman and McCaughan (6); these investigators reported a "continuous" fasting pancreatic secretion, not a "periodic" one.

Early in our investigations (7) we observed highly irregular pancreatic outflow in fasting dogs with fistulae of the Dragstedt (8) and Inlow (9) types. In the former fistula the flow was "continuous" with irregular periods of augmentation, while in the latter the pancreatic flow was irregularly periodic. Irregularities in the drop-by-drop pancreatic secretion were noted in both types of fistulae (Scott, 10). These irregularities in the rate-of-flow of pancreatic juice and appearance of periods of augmen-

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tation aroused doubts concerning the extreme regularity of the "periodic work" as reported by Boldyreff.

In previous experiments the type of gastric motility was not analysed and poor recording methods were used. It seems probable, however, that these investigators were inadequately recording low-grade gastric hunger contractions (Carlson, 11). Because of our experience in preparing many pancreatic fistulae (over 200) it was decided to re-investigate this problem employing better methods.

Methods. Inlow pancreatic fistulae, as modified by us (12), were prepared in vigorous dogs. In this fistula the major pancreatic duct was transplanted to the abdominal skin (the accessory ducts were undisturbed) which permitted easy cannulation at each experimental period. Animals were gastrostomized according to the method of Carlson (13). This gastrostomy did not leak and proved superior to the metal-cannula method. Following recovery from the pancreatic fistula and gastrostomy each animal was trained to lie quietly, 24 hours post cibum, on a padded table. Pancreatic juice was collected by a glass cannula, cemented into the transplanted duct with collodion, connected to an automatic drop counter and recorder. Intragastric pressure was recorded by a water manometer connected to a condom balloon which was inflated to a constant pressure of 5 cm. of water and introduced into the fundus of the stomach via the gastrostomy; a spring device inside the balloon (14) maintained the fundic position of the balloon and simplified its introduction. Fluid gastric content, when present, flowed out of a separate intragastric tube.

Observations were conducted for 2 to 6 hours on the trained animals which appeared comfortable and quiet throughout the experiment; these dogs often slept during the greatest part of the experiment. When a dog's health failed because of prolonged loss of pancreatic juice, the animal was sacrificed. All of these animals lost weight, some losing as much as 30 per cent of their preoperative weight.

Results. Ninety records of intragastric pressure and pancreatic secretion were obtained on 9 dogs. These records are summarized in table 1.

Records of group A were included because it was found that measurable quantities of pancreatic juice failed to flow from some fistulae, for periods often lasting for several days, followed by reappearance of pancreatic juice. No explanation has been found for this phenomenon, which seems to be a characteristic of this fistula. Thirty-two records, groups B and C, showed independent activity of the stomach or pancreas. Forty-eight records in group D evidenced both pancreatic secretion and hunger contractions with a high temporal correlation between them. Table 2 shows an analysis of these records.

Forty of these (83 per cent) showed hunger contractions and simultaneous augmentation of pancreatic secretion. Typical records will be noted

in figure 1. Eight records (17 per cent) revealed no apparent relationship between hunger and pancreatic secretion (see fig. 2). In these records hunger and secretion did not show a correlation, hunger appearing without secretion and vice versa.

The exact correlation between the peak-response of pancreatic secretion and the hunger period is shown in table 3, the hunger period being arbitrarily divided into three equal parts. It will be noted that pancreatic juice flowed most rapidly in the first two parts of the hunger period. However, rapid secretion was noted in the third part of the hunger period in a fair percentage of cases.

TABLE 1

Pancreatic secretion and hunger contractions in nine dogs having pancreatic fistulae and gastrostomies

GROUP	NUMBER OF RECORDS	RECORDS SHOWING PANCREATIC SECRETION	RECORDS SHOWING HUNGER CONTRACTIONS
A	10	0	0
B	24	24	0
C	8	0	8
D	48	48	48
Totals...........	90	72	56

TABLE 2

Analysis of records showing both pancreatic secretion and hunger contractions

GROUP	NUMBER OF RECORDS	CORRELATION BETWEEN HUNGER AND SECRETION	NO CORRELATION BETWEEN HUNGER AND SECRETION
D	48	40	8

Some records (table 1) exhibited a flow of pancreatic juice without an accompanying hunger period. These records were of interest because the rapid secretion often continued over a period of hours (fig. 3). Hunger contractions without accompanying pancreatic juice, seen in some records, was not attributable to accidental obstruction of the flow of juice because in all instances the cannula was examined to rule out such a possibility. We are certain that when pancreatic juice flowed from the duct, that flow was recorded accurately.

Discussion. The foregoing results show clearly that our experiments did not reveal the close relationship between gastric hunger periods and external pancreatic secretion claimed by Boldyreff. Of eighty records showing either pancreatic secretion or hunger or both, exactly fifty per cent revealed simultaneous periodic activity of these two organs.

In addition, we have shown that copious pancreatic secretion under fasting conditions can occur in the complete absence of gastric motility

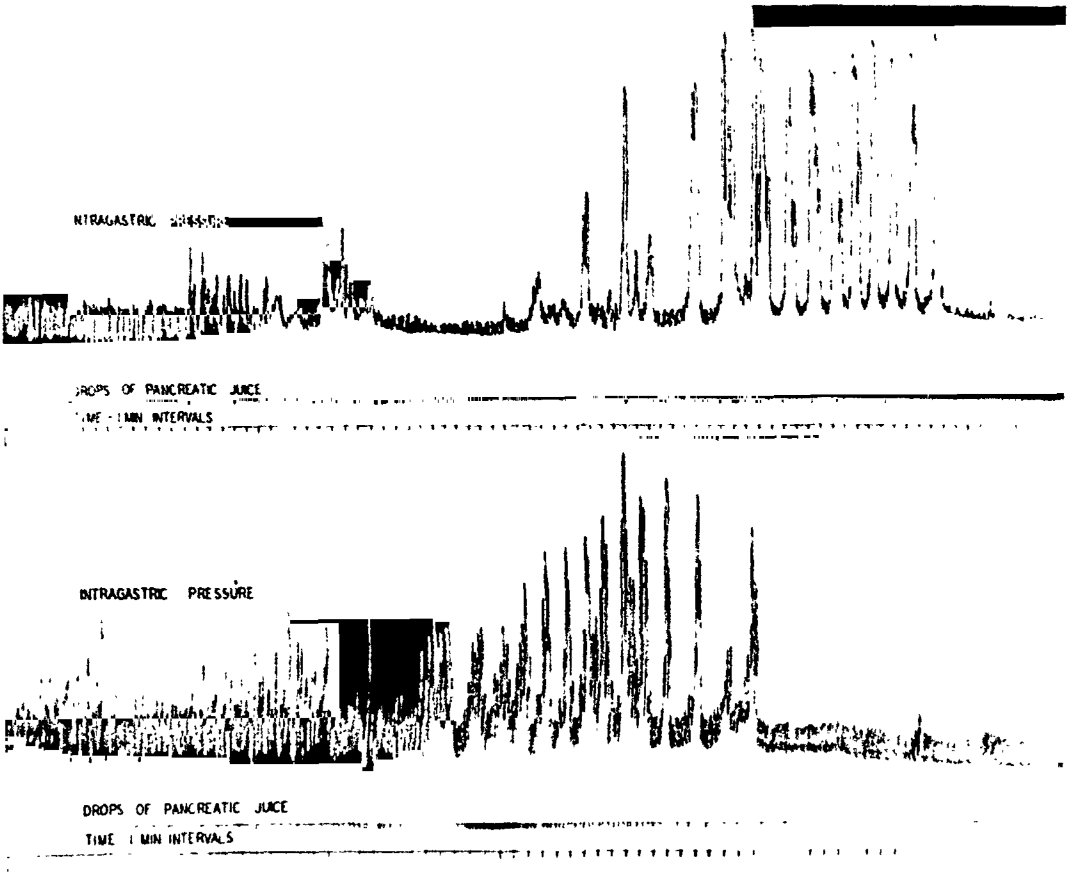

Fig. 1. Pancreatic secretion and gastric hunger contractions which show a temporal correlation.

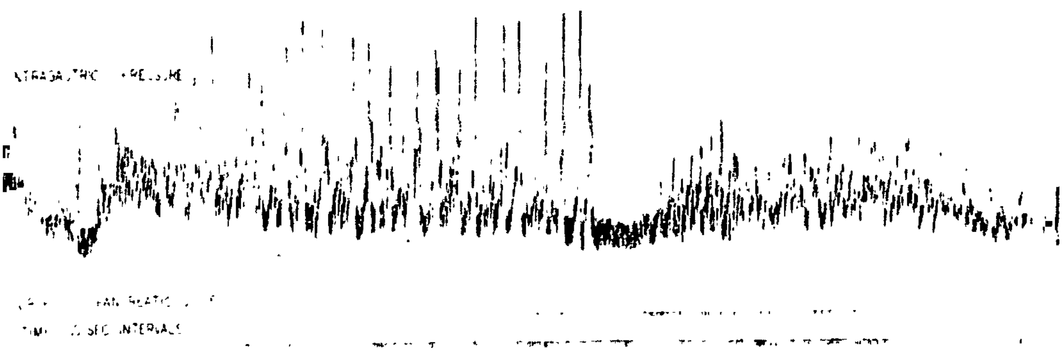

Fig. 2. Record of pancreatic secretion and intragastric pressure which shows no correlation between the activity of the stomach and pancreas.

(27 per cent of the records). Also, in nine per cent of the experiments, no pancreatic juice was secreted but strong hunger occurred. Boldyreff

reported that the periodic activity of one organ was always accompanied by simultaneous activity of the other digestive organs studied. This was not true in our animals, even though the periodic activity of any one organ was very strong. Furthermore, nine per cent of all records showed no apparent correlation between pancreatic secretion and hunger motility although the periodic activity of both organs occurred during the experiment.

These results are significant in view of Boldyreff's reports that the periodic activity was exceptionally regular. He stated that he could outline his program for an entire day's work, performing other work according to clock schedule during the "rest" periods between the periodic activity of the digestive organs. We have never observed such regularity of the

TABLE 3

Relation of the peak-response of pancreatic secretion to the hunger period

PART OF HUNGER PERIOD	NUMBER OF RECORDS
First	15
Middle	14
Last	6
Indeterminable	5

Fig. 3. Rapid pancreatic secretion unaccompanied by gastric hunger contractions.

periodic activity. An attempt to classify data according to the length of the active periods and the periods of rest was completely unsuccessful because pancreatic secretion and gastric motility were absolutely irregular, even in the same animal on the same day. Indeed, pancreatic secretion was often almost continuous. We can offer no explanation for the variation between our results and those of Boldyreff except that his animals had combined pancreatic and intestinal fistulae. It is possible that Boldyreff missed or ignored small amounts of pancreatic juice because of the type of fistula used.

From table 3 it appeared that the peak-response of pancreatic secretion and a particular part of the hunger period did not bear any constant relationship. It is true that the greatest volume-flow of pancreatic juice occurred most frequently in the earlier parts of the hunger period, but it was not uncommon for the peak-response to occur in the terminal portion

of this period. Just why there should be such variation is unknown and may be of no particular significance. The analysis is of interest, however, because it shows that activity of one organ does not invariably call forth the activity of the other immediately. This observation does not strengthen the belief that the activity of the two organs is controlled by a single mechanism.

Since one-half of our records (not including records in group A, table 1), showing pancreatic secretion and gastric motility, revealed a definite relationship between the two, it seems reasonable to believe that such observations are not pure coincidence; when both organs were active the correlation was 83 per cent. The mechanism responsible for this correlation has not been found.

Many possible explanations might be suggested but little concrete evidence has yet been advanced. E. B. Boldyreff (4) reported that during periods of fasting pancreatic secretion the blood-glucose level was lowered which was followed by an elevation of this level in the subsequent "rest" period. Additionally, he stated that prolonged stoppage of the pancreatic ducts produced mild diabetes mellitus. Mulinos (15) and W. W. Scott (16) reported no direct causal relation between hunger periods and the blood-glucose level. Dragstedt (8) found no evidence of diabetes mellitus in dogs with total pancreatic fistulae. Scott (17) has, in a few experiments, found inconstant blood-glucose fluctuations in dogs with pancreatic fistulae. Marked hypoglycemia following insulin is accompanied by an augmentation of pancreatic secretion and gastric motility. Some evidence (incomplete at present) indicates that the mechanisms of concomitant augmentation are not identical since vagotomy abolished the response of the latter to insulin without affecting the former. From our results it seems probable, therefore, that the relation between the pancreas and stomach is not the simple one postulated by Boldyreff.

Periodic pancreatic outflow is not the result of motility of the pancreatic ducts because the amount of this secretion usually exceeded the volume which could be held by the pancreatic ducts.

CONCLUSIONS

1. A temporal correlation exists between the periodic motility of the stomach (hunger) and the external secretion of the pancreas.
2. The correlation of this periodic activity was not always present since only 50 per cent of all records showed simultaneous activity of the stomach and pancreas.
3. When both fasting pancreatic secretion and hunger periods were present the correlation of this activity was 83 per cent.
4. Periodic activity of the stomach and pancreas occurred at irregular intervals and not with the regularity claimed by Boldyreff.

5. Fasting pancreatic secretion can occur in complete absence of hunger contractions and can fail to appear with the onset of powerful hunger contractions.

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THE EFFECT OF CHANGES IN THE CALCIUM CONTENT OF THE CEREBROSPINAL FLUID ON SPINAL REFLEX ACTIVITY IN THE DOG[1]

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Although the cerebrospinal fluid system has been the subject of extensive study, most workers have concerned themselves mainly with the mechanics of the formation and reabsorption of this fluid, relatively little attention having been paid to the relationships existing between the cerebrospinal fluid and the tissue fluid of the brain and spinal cord. The probability of free communication between the subarachnoid space and the tissue spaces of the nervous system has been demonstrated by Mott (1910), Weed (1914), and others. If such communication does exist, it is reasonable to assume that any change in the composition of the cerebrospinal fluid will be reflected by a similar change in the composition of the interstitial fluids, and thus possibly by modified activity of the neurones bathed by the latter.

Several workers have reported spontaneous neuromuscular activity following the intrathecal administration of various solutions. Collip (1920) observed tetanoid behavior in dogs following the introduction of small volumes of $NaHCO_3$, hypertonic NaCl and KCl, and Na_2HPO_4 into the lumbar subarachnoid space. $CaCl_2$ antagonized this motor activity, and it was concluded that the tetany was due to a disturbance in the concentrations of the various cations, especially of calcium. Huggins and Hastings (1933) observed that the injection of sodium citrate into the cisterna magna of the dog produced motor excitation which was antagonized by calcium chloride. Calcium citrate provoked no motor response. Mullin, Hastings, and Lees (1938) produced tetany by the cisternal injection of salt solutions which were ionically balanced except for the absence of calcium. Similar results were obtained with the injection of sodium citrate. Bathing the lower spinal cord with the calcium-free solution was ineffective, although here too citrate produced spontaneous neuromuscular activity.

[1] A portion of these data was presented as a preliminary report at the New Orleans meeting of the American Physiological Society in April, 1940.

METHODS. The action of low calcium solutions on the spinal cord has been reinvestigated using a method of continuous perfusion through the spinal subarachnoid space at constant pressure and temperature (Merlis and Lawson, 1939). Dogs were anesthetized with sodium barbital (0.25 gram/kgm. intravenously), the spinal cord was sectioned at T10, and the caudal segment of the cord was prepared for perfusion. As an indicator of the effect of changes in the calcium content of the perfusion fluid on the activity of simple functional neural units, the flexion reflex of the tibialis anticus muscle was elicited by stimulating the posterior tibial nerve at 5 to 8 second intervals with single shocks applied from a thyratron stimulator. The tension developed by the muscle was recorded on smoked paper by means of a torsion wire myograph.

The control perfusion fluid was an artificial cerebrospinal fluid of the following composition (moles/liter): Na—0.141, K—0.0033, Ca—0.00125, Mg—0.0012, Cl—0.152, HPO_4—0.00048, HCO_3—0.021, glucose—0.0034, urea—0.0022. A change in the calcium concentration was always compensated by an opposite change in NaCl concentration so as to have control and test solutions isosmolar. All solutions were brought to a pH about 7.4 with CO_2, using phenol red as the indicator.

The rate of flow through the spinal subarachnoid space was varied either by changing the perfusion head of pressure, or by maintaining a constant pressure head and partially obstructing the outflow by means of a constriction. Rates varying from 2 to 25 cc./minute with perfusion pressures of 8 mm. Hg or higher were used.

RESULTS. The subarachnoid perfusion of the balanced artificial cerebrospinal fluid had, in most cases, no effect on the flexion reflex. Occasionally a slight diminution of the reflex was apparent, but augmentation was never seen. When the calcium-free solution was substituted for the balanced solution, there was marked augmentation of the reflex, an increase in the tone of the muscle, and spontaneous twitching of the muscles of the lower half of the body (fig. 1). These effects usually appeared in from 1 to 5 minutes, at least a portion of the latency being accounted for by the 3 cc. dead space between the fluid reservoirs and the spinal subarachnoid space. Replacement by the balanced salt solution was followed by subsidence of these effects, usually within less than 5 minutes.

Sodium citrate (0.1–5.0 per cent) intrathecally was followed by similar motor activity, which was more intense than that seen with calcium-free perfusions.

Solutions containing an excess of calcium, in concentrations as high as four times the control value, had no demonstrable effect on the flexion reflex. Higher concentrations than these were not studied.

The effects of a calcium-free perfusion appeared to be more intimately connected with the rate of flow through the spinal subarachnoid space than

with the perfusion head of pressure. Successful perfusions were obtained with pressures as low as 8 mm. Hg and as high as 100 mm. Hg. The pressures were always kept well below mean carotid pressure to avoid the possibility of marked reduction in blood flow through the cord with consequent anoxic effects. With perfusion pressure constant, an increase in the rate of perfusion produced increased effects (fig. 2).

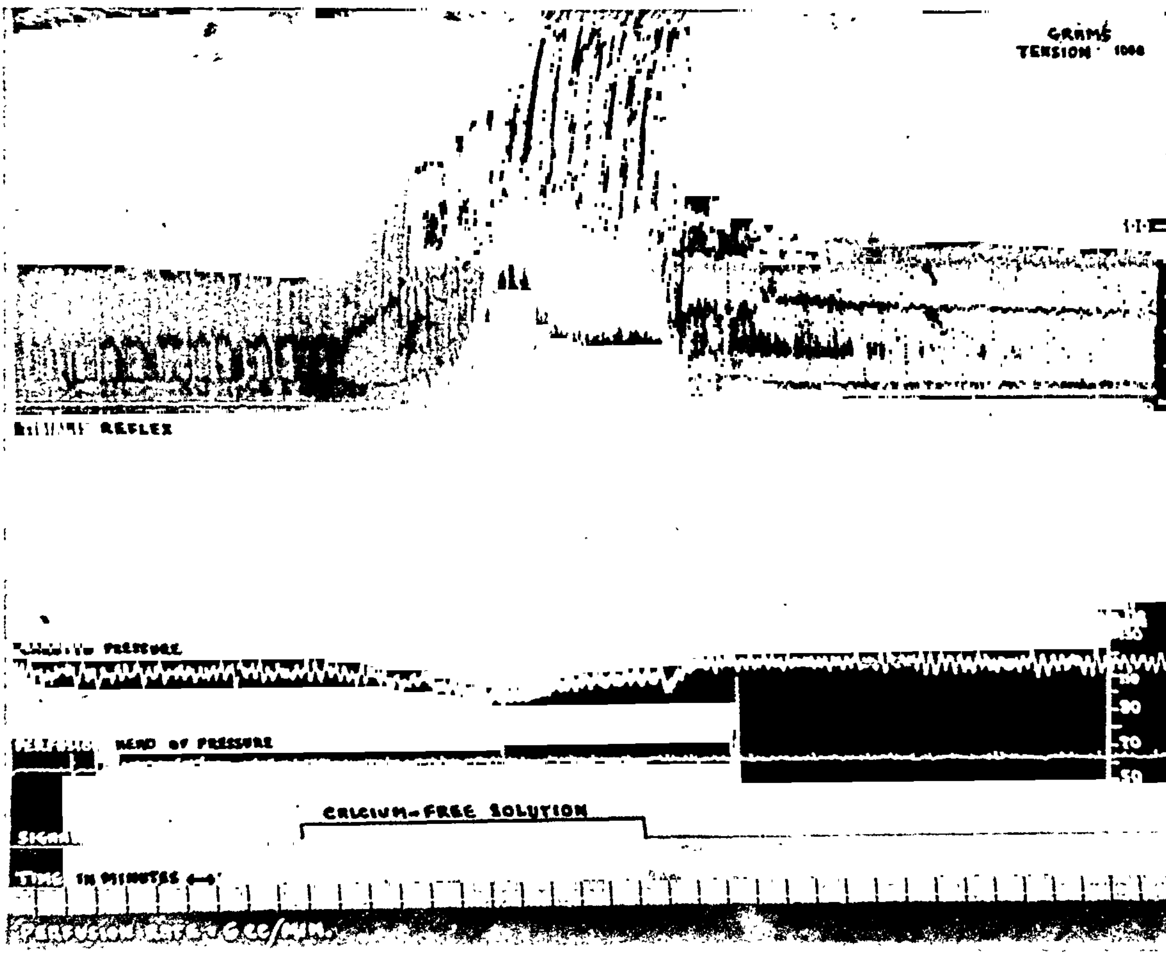

Fig. 1. The effect of a calcium-free perfusion. Irregular fibrillary contractions are recorded in the intervals between reflex contractions during the response.

Discussion. The failure of Mullin and his collaborators (1938) to produce tetany by bathing the spinal cord with low calcium solutions may be attributable to the method employed in their studies. If the action of these solutions is due to a lowering of the calcium content of the fluids bathing the cells of the central nervous system, it is essential that a diffusion gradient of sufficient magnitude be set up between the subarachnoid fluid and the interstitial fluids. Simple bathing of the exposed spinal cord with small volumes of salt solution might very well fail to achieve this end. The

effectiveness of the method of continuous subarachnoid perfusion may be ascribed to maintenance of an effective gradient.

It has been shown by Lehmann (1937) and by Brink and Bronk (1938) that a decrease in the calcium content of the fluids bathing peripheral nerve produces increased excitability of the nerve fibers and may provoke

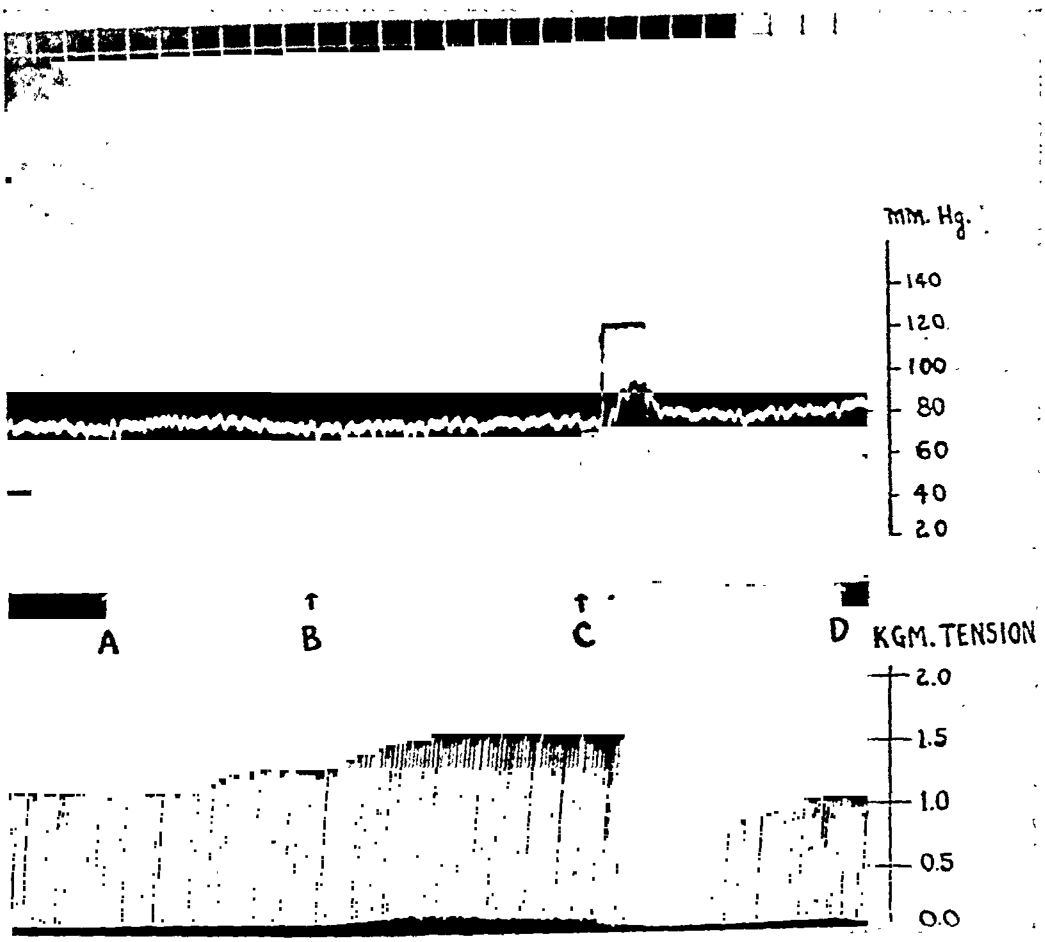

Fig. 2. Tracings from above downward: 1, time in minutes; 2, carotid pressure; 3, perfusion head of pressure; 4, signal; 5, tibialis anticus reflex. At *A*, calcium-free perfusion begun, perfusion rate: 3.4 cc./min. At *B*, perfusion rate increased to 16 cc./min. At *C*, perfusion pressure raised above carotid pressure. At *D*, calcium-free solution replaced by balanced salt solution. Fibrillary contractions are just visible in the intervals between reflex contractions during the Calcium-free perfusion, and disappear with the reflex during cord ischemia at *C*.

spontaneous discharge in many of them. The tetany produced by the spinal subarachnoid perfusion of calcium-free solutions might be due to an action of these fluids not in the cord itself but rather on the ventral rootlets in their intrathecal course. If this were true, synaptic transmision through the cord would not be essential, at least insofar as the spontaneous

twitching is concerned. This possibility has been tested by blocking synaptic transmission through the medium of the ischemia which is produced when perfusion pressure is raised above blood pressure (Luckhardt and Montgomery, 1929; Merlis and Lawson, 1939). When synaptic transmission was abolished by such a procedure, not only was the flexion reflex abolished, but so too were the spontaneous twitchings (fig. 2). It is therefore apparent that the muscular twitchings are not caused by spontaneous firing of the motoneurones of the cord, or of the nerve fibers of the anterior roots.

Two possibilities still remain: 1, the action is on the afferent limb of the reflex arc, i.e., spontaneous discharge of the dorsal roots or internuncial neurones, or 2, there is no spontaneous firing, but rather an increase in the excitability of the cord neurones, so that they respond more effectively to the normally incident flow of afferent impulses arising in the periphery. When the dorsal roots of the perfused cord segments were sectioned extradurally, spontaneous twitching was completely abolished, although synaptic transmission was still possible as was shown by eliciting reflex responses by mechanical stimulation of the central ends of the cut dorsal roots. This can mean only that the spontaneous tetanic manifestations depend upon the receipt of afferent impulses from the periphery and are not due to spontaneous firing of neurones of the cord, or of dorsal or ventral root fibers.

In the case of citrate perfusions, the situation is somewhat different. Ischemic blocking of synaptic transmission through the cord diminished, but did not abolish, the twitching which results from a citrate perfusion. Citrate, therefore, does cause spontaneous discharge of the motoneurones of the cord, or of the intrathecal ventral root fibers, or of both. Corroborative evidence was obtained from the deafferented preparation, where again there was diminution, but not abolition of twitching.

It is of interest to note the parallelism between the tetanic manifestations produced by calcium-free cerebrospinal fluid and those reported by West (1935) in his studies on parathyroid tetany. The results of his experiments led West to conclude that there were three neuromuscular manifestations of parathyroid tetany: fibrillary twitching, tonic, and clonic contractions. The clonic and tonic contractions were abolished by deafferentation of the cord, although the fibrillary movements were still in evidence after this operation. This finding is quite similar to that reported in this study, in which the tetany, although definitely central in origin, does depend upon the integrity of the somatic reflex arcs before it may be manifested.

SUMMARY AND CONCLUSIONS

1. The effect of changes in the calcium content of balanced salt solutions perfused through the lower spinal subarachnoid space at constant pressure

and temperature was studied in barbitalized dogs with spinal cord sectioned at T10.

2. Calcium-free solutions produced an augmentation of the spinal flexion reflex, an increase in muscle tone, and spontaneous twitching of the muscles of the lower half of the body.

3. The tetany produced by calcium-free perfusions is not due to spontaneous firing of the motoneurones nor of the dorsal or ventral rootlets. The twitching requires the integrity of the spinal reflex arcs, for it disappears when these arcs are broken. It appears to be due to an increased responsiveness of the cord neurones to the normally incident afferent impulses from the periphery.

4. Similar motor activity was seen with sodium citrate perfusions, differing in that the twitching persisted, with diminution, when synaptic transmission was abolished. Citrate appeared to cause spontaneous firing of the motoneurones, or of the dorsal and ventral rootlets, or both.

5. High calcium solutions, up to four times normal concentrations, were without effect.

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A SYNDROME OF POLYDIPSIA AND POLYURIA INDUCED IN NORMAL ANIMALS BY DESOXYCORTICOSTERONE ACETATE

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Desoxycorticosterone has been shown to have a marked effect upon the renal excretion of sodium. The administration of this hormone to patients with Addison's disease or to dogs in uncompensated adrenal insufficiency results in a retention of sodium which under certain circumstances may be associated with a striking accumulation of extracellular fluid and symptoms of congestive heart failure (1, 2). We have not been able to produce these symptoms in normal animals. When normal dogs are given 25 mgm. of hormone in daily injections there is no striking accumulation of extracellular fluid and no congestive heart failure. Instead the normal animals develop a syndrome resembling that of diabetes insipidus, as might have been anticipated from the earlier work of Teel (3), Wermer (4), and also Silvette and Britton (5).

Experimental. The evolution of the syndrome can be seen in the accompanying chart of a normal female dog (fig. 1). This was an 11 kgm. animal kept in a metabolism cage, given one and one-quarter pounds of fresh raw lean meat each day, a haliver oil capsule, and all the water she wanted to drink. Throughout the experiment the urinary excretion of sodium, chloride, potassium, and nitrogen was measured in five-day periods. At the beginning of each period the blood serum was analyzed for sodium, potassium, and total protein. The data are presented in table 1 and figure 1.

For the two weeks before hormone injections were given, the animal's water intake averaged 400 cc. a day. The urine volume was also about 400 cc. a day with a specific gravity of 1.045. When subcutaneous injections of desoxycorticosterone acetate were begun, the effect upon the intake and output of fluid was striking. At the end of six weeks the dog was drinking not 400 cc. but 1000 cc. a day. A similar change occurred in the urine volume which also increased to about one liter a day with a specific gravity down to 1.025. These changes were not accompanied

by any evidence of excessive fluid retention or congestive heart failure. The balance studies (table 1) indicated that no great retention of sodium had occurred although the serum sodium concentration was consistently elevated. The serum protein concentration was essentially unaltered. The dog had gained slightly in weight probably because $1\frac{1}{4}$ pounds of meat a day was more than her maintenance requirement. This gain of weight had commenced before the injections of hormone were begun.

At the end of seven weeks, the meat was reduced to three-quarters of a pound a day, the injections were continued, and in addition to the hor-

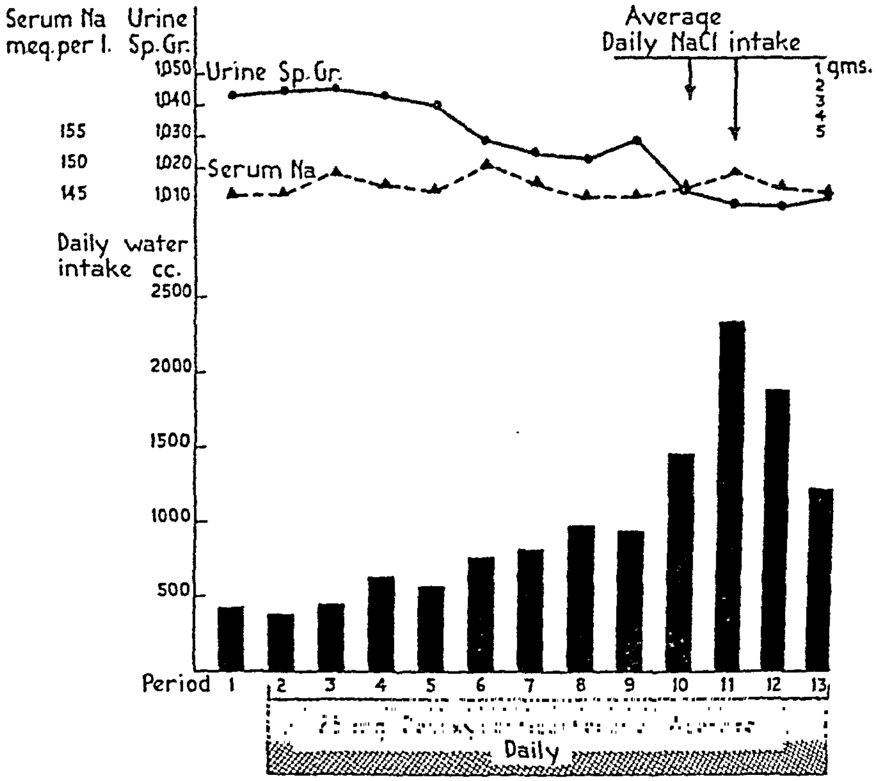

Fig. 1. Evolution of polydypsia in course of treatment of normal dog with desoxycorticosterone acetate.

mone the animal was given 40 to 80 m.eq. of sodium chloride a day in her drinking water. The addition of this amount of sodium chloride to the regime resulted in a further increase in water intake accompanied by an equal increase in urine output and a further decrease in urine specific gravity down to a level of about 1.008. After one week on the high salt regime the animal developed a peculiar muscular weakness which has been seen in all five of our dogs on similar regimes and has been previously described (6). The administration of sodium chloride was stopped and the volume of fluid exchange returned to about 1 liter a day.

The effect of potassium chloride was then studied. Thirty to 80 m.eq. of potassium chloride were administered to the animal of figure 1 each day for a period of ten days. No sustained effect upon water exchange was demonstrable although the specific gravity of the urine rose to 1.015. Potassium chloride was stopped, sodium chloride was again administered, and the usual effects of salt were observed: increase in fluid intake, increase in urine output, decrease in urine specific gravity.

In another dog we have studied the effect of pitressin upon this syndrome of polydipsia and polyuria. Figure 2 is the chart of a normal male animal in which the syndrome had been well established by the administration of 8.5 grams of sodium chloride and 25 mgm. of hormone each day for several weeks.

TABLE 1

Balance studies of normal dog treated with desoxycorticosterone acetate

PERIOD	WEIGHT	AVERAGE DAILY INTAKE					AVERAGE DAILY OUTPUT						BLOOD AT END OF PERIOD					
													Serum					
		Meat	Desoxycorticosterone acetate	NaCl	KCl	Water intake	Urine volume	Sp.G.	N_2	Na	K	Cl	Na	K	Cl	Protein	Plasma NPN	Blood sugar
	kgm.	*lbs.*	*mgm.*	*m.eq.*	*m.eq.*	*cc.*	*cc.*		*grams*	*m.eq.*			*m.eq. per l.*			*grams*	*mgm. per cent*	
1	10.9	1.25	0	0	0	425	415	1043	15.5	6.5	43.5	4.6	145	4.4	107	6.7	31	77
2	11.3		25	0	0	395	365	1046	16.8	4.0	44.6	5.6	146	4.1	109	6.2	35	78
3	11.5					445	420	1047	16.5	6.0	46	7.2	149	3.9	107	6.5	29	77
4	11.7					615	485	1044	16.1	5.4	48	8.3	148	3.8	107	6.1	33	82
5	11.9					580	675	1041	16.5	5.4	48.2	9.1	147	3.3	105	6.1	33	77
6	12.0					760	805	1029	17.8	6.6	49	9.1	151	3.3	107	6.6	24	69
7	12.2					810	810	1026	17.7	5.9	43	6.8	148	3.2	104	6.6	29	74
8	12.4					975	1010	1023	17.7	11.5	44.8	9.6	146	3.1	103	6.5	28	80
9	12.4					925	940	1028	17.2	10.4	42.4	8.7	147	3.5	102	6.6	31	77
10	12.1	0.75	25	38.8	0	1490	1445	1014	10.6	43.3	34.2	45	147	3.3	107	6.3	23	74
11	12.0			85.6	0	2330	2250	1009	10.1	79.8	32.6	85	150	2.9	107	6.3	20	74*
12	11.8			0	0	1875	1800	1008	10.5	12.4	21.2	8.7	148	3.2	100	6.4	27	76
13	11.8			0	0	1280	1215	1011	11.0	9.0	23.8	5.6	147	3.2	101	6.7	23	80
14	11.8			0	33.8	1535	1490	1009	11.1	16.7	36.8	32.2	147	4.0	109	6.3	24	71
15	11.6			0	79.0	1420	1290	1014	9.9	13.9	95.3	79	148	4.1	111	6.5	27	111
16	11.5	0.75	25	81	0	1930	1835	1010	10.7	78.0	48.4	90.2	147	3.2	106	6.8	23	70
17	11.6			51	0	1720	1500	1010	10.0	40.9	25.0	46.0	147	2.4	108	5.9	20	72*
18	11.8			107	0	2910	2720	1006	10.7	90.0	35.5	102	152	2.7	115	6.4	28	85†

* Paralysis.

† Paralysis and death.

Pitressin subcutaneously in rather large doses caused some decrease in the fluid exchange with a slight decrease in the serum sodium concentration and a slight increase in the urine specific gravity. Restriction of the animal's intake to two liters for one day resulted in a sharp rise in the serum sodium concentration and an increase in the urine specific gravity. Following this restriction, there seemed to be a compensatory increase

in water intake to 9000 cc. with a comparable increase in urine volume and a resultant drop in urine specific gravity and serum sodium concentration.

The injections of hormone were then stopped but the administration of sodium chloride was continued. Despite continuance of salt administration there was a prompt cessation of polydipsia and polyuria. In the course of seven days the urine specific gravity rose to normal, the water exchange fell from seven liters down to one and one-half liters a day, the serum sodium concentration remained about the same.

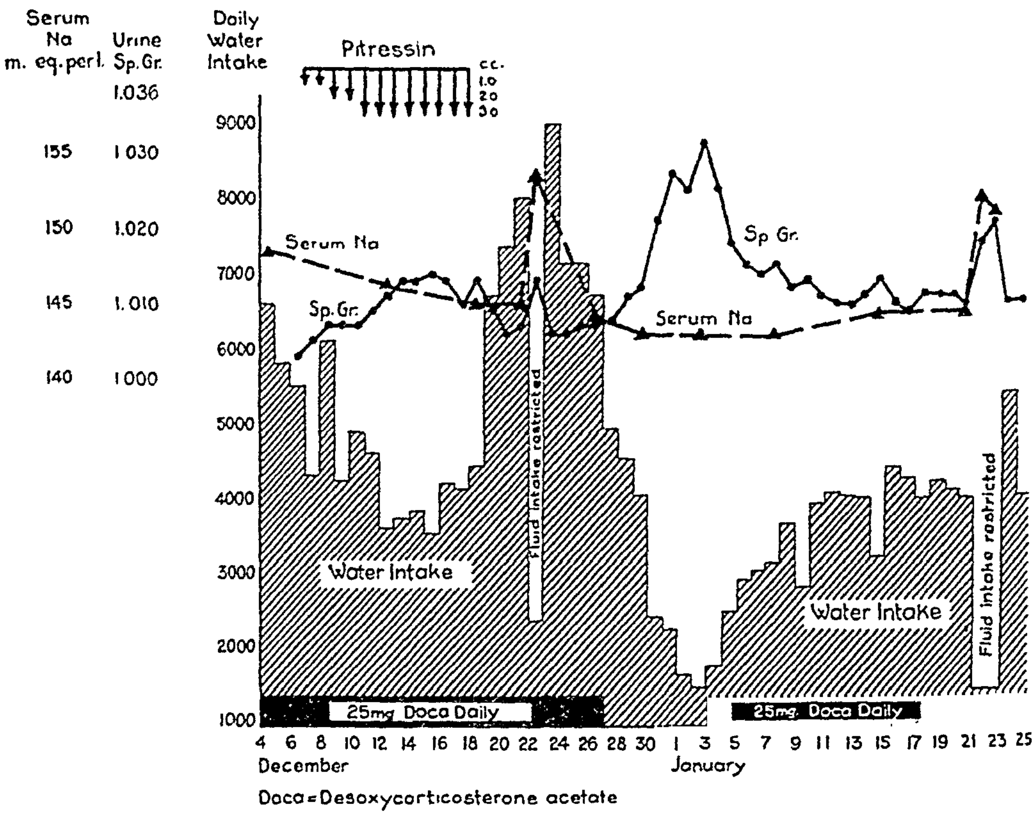

Fig. 2. Effects of pitressin, fluid restriction and withdrawal of desoxycorticosterone acetate upon the polydypsia produced in a normal dog by desoxycorticosterone acetate and sodium chloride.

The readministration of hormone resulted in reappearance of the entire syndrome with increased water consumption, increased urine volume, and decreased urine specific gravity.

When the syndrome was again well established, the intake of fluid was restricted for two days. Serum sodium concentration and urine specific gravity rose sharply and the animal was obviously thirsty and very uncomfortable although he did not go into negative fluid balance or show significant evidence of dehydration such as loss of weight or increased

serum protein concentration. When free access to fluid was again allowed, there was a sharp increase in fluid intake with a concomitant drop in serum sodium concentration and urine specific gravity.

Observations similar to those which have just been described were made upon three other normal dogs, two females and one male. The male animal was killed with chloroform and autopsied when the symptoms of diabetes insipidus were well established. The adrenal glands of this animal were somewhat small but the other organs and the microscopic sections of the pituitary gland, kidneys, adrenals, and gonads were essentially normal. It is interesting that none of the normal animals developed symptoms of adrenal insufficiency when the injections were stopped.

We were not able to produce polyuria and polydipsia in normal rats on a low salt, low potassium diet, although enough hormone was used to produce an atrophy of the adrenal glands. In normal adult male rats kept on a high salt diet, we were able to double the water intake by giving the animals 5 mgm. of hormone daily. The hormone-treated animals drank 300 cc. of water a day and had an average urine specific gravity of 1.015. The control rats that were given a high salt diet and no hormone drank 150 cc. of water a day and had a urine specific gravity of 1.022.

Discussion. It is tempting to speculate upon the mechanism by which desoxycorticosterone acetate produces polydipsia and polyuria in normal animals. Although the syndrome superficially resembles that of diabetes insipidus the two states are dissimilar in at least two important respects: pituitrin is relatively ineffective and fluid restriction does not cause dehydration in our animals. The converse is true in diabetes insipidus. It seems probable, therefore, that the condition which we have described is primarily a thirst and only secondarily a polyuria. The cause of the thirst is not clear, but it may be related to the maintained increase in extracellular sodium concentration or to some associated disturbance of the osmolar balance between extracellular and intracellular fluid. Whatever the mechanism of the polydipsia may be, the consequent polyuria enables the normal dog to avoid excessive retention of sodium. A rapid flow of urine through the tubules would tend to counteract the increased sodium reabsorption brought about by the adrenal cortical hormone.

CONCLUSIONS

Large doses of desoxycorticosterone acetate induce in normal animals a syndrome of polydipsia and polyuria similar to that seen in diabetes insipidus.

The mechanism of this effect and its differentiation from diabetes insipidus are discussed.

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HEAT EXCHANGE AND REGULATION IN RADIANT ENVIRONMENTS ABOVE AND BELOW AIR TEMPERATURE

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Object of study. In previous contributions from this laboratory (Herrington, Winslow and Gagge, 1937; Winslow, Herrington and Gagge, 1938) data have been presented with respect to the reactions of the human body to atmospheric conditions in which air and wall temperatures were independently varied. Our results suggested that a cold-air warm-wall situation exhibited unexpected phenomena, the skin temperature of the subjects being lower than in the case of exposure to equal wall and air temperatures having, physically, an equivalent cooling effect.

In these earlier experiments, our high radiation was produced by reflection from the surfaces of a copper booth in which the subject under observation was placed; and we had no means of studying the influence of cold walls with warm air. Also, air movement was not recorded in each experiment but assumed from preliminary tests. With the construction of a new experimental booth designed on a different principle, it has been possible to study both the cold-air hot-wall situation and the hot-air cold-wall situation under comparable conditions and to pay special attention to air movement.

Description of the new experimental booth. The new booth is octagonal in shape, each of its eight walls being four feet wide and eight feet high and the distance across the booth between the centers of each pair of opposite panels being ten feet. Each panel consists of a steel frame supporting a four by six foot copper tank of one inch interior thickness through which may be circulated a "brine" solution of di-ethylene glycol and water. The temperature of the panels can be reduced to −6°C. by passing the solution through an ammonia brine cooler or can be raised to 72°C. by passing the solution through a gas-fired water-heating boiler. Alternate panels are hinged so that they can be swung outward, and the "brine" solution is supplied to these movable panels by short lengths of rubber hose. The interior face of the panels is painted black for maximum emissivity from the radiant surfaces, and the floor and ceiling of the booth are of aluminum to increase reflectivity from those surfaces which are not directly heated or cooled.

The booth is placed in the center of a seventeen-foot square shell of steel, and this shell, in turn, is inside a twenty-foot square room insulated with six inches of cork on all six surfaces. Conditioned air is introduced from a York conditioning unit which can supply air at any temperature between −18 and +60°C. with an accuracy of ±0.5°, and can control the relative humidity within 2 per cent over a dry-bulb range between 5° and 60°C., and within the limits of ten per cent and ninety per cent of saturation.

The conditioned air enters the insulated room near the ceiling and is directed by dampers into the steel shell, upward or downward, along all its four sides. The booth itself has dampers above and below the radiant panels and the air introduced into the steel shell passes upward or downward through the booth, depending on panel temperature. The actual air velocity within the booth can be controlled by the upper and lower dampers and is ordinarily maintained at a level of about seven centimeters per second.

Conduct of experiments. The new experiments here reported were all performed with the same two young male subjects (VII and IX) used in earlier experiments, unclothed (except for an athletic supporter) and in a semi-reclining position. At the beginning of an experiment, the subject is seated in a movable chair (with an aluminum frame and canvas back and seat). In this chair he remains for one hour in an equalizing chamber, maintained, in all cases, at a temperature between 30° and 31°C., with a relative humidity of 40 to 50 per cent and an air movement of approximately ten centimeters per second. After this preliminary treatment, which produces a reasonably standard thermal adaptation, the chair is wheeled into the booth and transferred by jacks to the platform scale used for measuring evaporation. The standard period of observation in the booth has been 240 minutes, in the major experiments here reported.

Air temperature and relative humidity within the booth are automatically recorded throughout. Mean radiant wall temperature is observed before the subject is brought in by the use of the Vernon globe thermometer placed in the center of the booth; and this measurement is correlated with the temperature of the brine within the panels, the latter temperature being recorded throughout the experiment. The brine temperature, which can be measured with high accuracy, is used in our final analysis for computing wall temperature on the basis of the established correlation between this reading and that of the globe thermometer. Air movement is measured by the hot-wire anemometer at the close of each experiment, with the chair present but without the subject, and at vertical levels corresponding to the position occupied by the head, chest, and calf of the subject when in the chair. The reasons for making this measurement without the presence of the subject have been outlined in a previous communication (Winslow, Gagge and Herrington, 1939).

During the course of an experiment, the following routine observations were made:

a. Rectal temperature, every 15 minutes, by a thermocouple inserted 14 cm. into the rectum and recorded on a potentiometer in the control room.

b. Skin temperature at 15 representative points on the body surface (Winslow, Herrington, and Gagge, 1936) measured every half hour by a Hardy thermopile held one centimeter from the body surface. Before and after each series of readings a calibrating observation was taken from two thermostatically controlled reference baths maintained at approximately 8°C. differential and including the skin temperature range observed.

c. Metabolism every 45 minutes by application of the Benedict-Roth apparatus. The subject breathed for the usual 6-minute interval in connection with an oxygen stream driven by motor-circulation through soda-lime and connected with a respirometer acting as an oxygen reservoir. The recording apparatus was in the control room.

d. Evaporative heat loss determined every half-hour by readings of weight loss on the platform scale upon which the subject sat.

Measurement of radiation area. Of the five factors in thermal interchange, metabolism and evaporation are, of course, observed directly. To estimate radiation interchange we must know not only the mean skin temperature of the subject and the mean radiation temperature of surrounding surfaces but also the radiation area of a given subject in a given position.

To determine this point we made a special series of experiments in which convection interchange was practically eliminated by holding air temperature approximately the same as skin temperature. Under such conditions, the algebraic sum of metabolism, evaporation, and storage should show a linear relation to the radiant heat interchange, as computed by the Stefan-Boltzmann Law from the observed mean skin temperature and the mean radiant wall temperature (Gagge, 1936).

The result of this relationship is indicated in figure 1. In computing the ordinate data we obtained storage values by assuming that change in total tissue temperature could be determined from mean change in skin temperature and rectal temperature between 40 and 200 minutes, giving the skin temperature a weight of one and rectal temperature a weight of two.

It will be seen from figure 1 that under neutral and warm conditions, the linearity law holds, and the line passes through the origin, thus validating the basic measurements involved. Under cold conditions, however, the linearity relation fails, indicating that our method of estimating storage (by a 1:2 weighting of skin and rectal temperature) was not justified. We shall return to this question in a later paragraph. Under extremely

hot conditions a deviation is again observed as a result of excessive sweat secretion, which drops off without cooling the body.

Where the linearity relationship does hold, the slope of the graph gives us the radiation area. For subject VII, this value is 1.47 sq. m. or 73 per cent of his DuBois area; for subject IX, it is 1.19 sq. m., or 74 per cent of his DuBois area.

In earlier studies (Gagge, Winslow and Herrington, 1938) we found a higher value for subject VII and a somewhat lower value for subject IX. The weight of subject VII changed materially during the interim and habitual posture maintained in the chair may also have influenced the

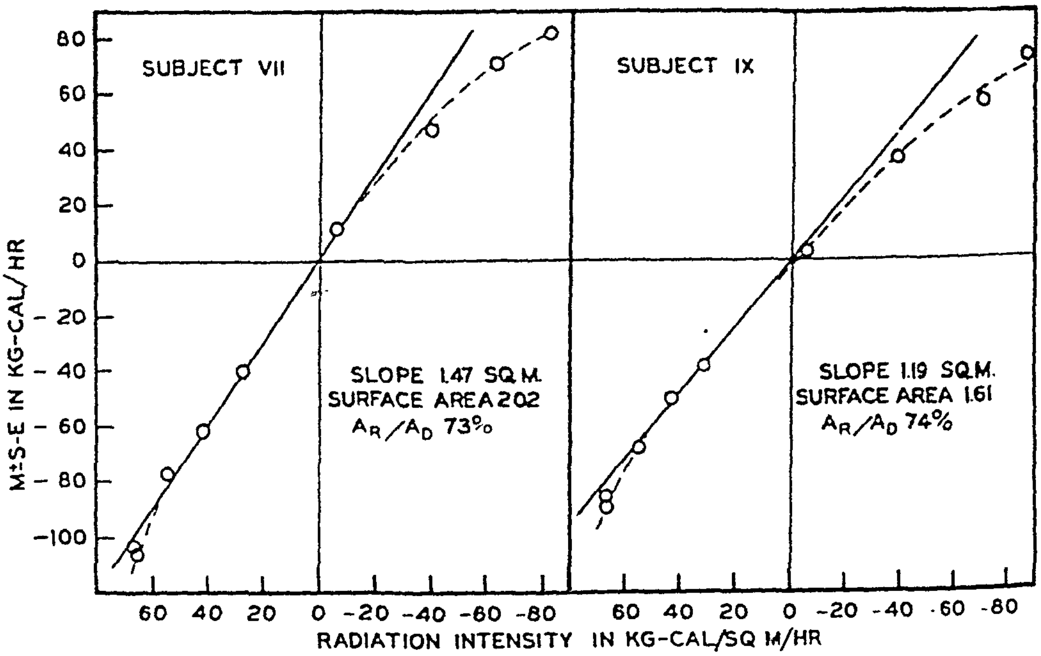

Fig. 1. Graph of heat exchange plotted against radiation intensity for determination of radiation area (with mean skin temperature equal to air temperature).

radiation area. We are inclined to believe that 70 to 75 per cent of the DuBois area is a representative value.

Determination of the convection constant. Having found the radiation area for each subject, our next problem was, of course, to determine the respective convection constants: and this has been done in a different way from that previously employed. In our earlier work, we computed K_C for a standard air movement of eight centimeters per second, which we assumed to hold approximately throughout the experiments. In the present series, the air movement was measured at the end of each experiment. We have shown elsewhere (Winslow, Gagge and Herrington, 1939) that air movement increases convective cooling in proportion to the square of its velocity (within the range of air velocities involved). With our new

data it is now possible to obtain a basic K_C for unit air velocity. For this purpose, we have again computed storage from mean changes in skin temperature and rectal temperature between 40 and 200 minutes, as before, weighting these two changes in the ratio of 1:2. We then (fig. 2) plotted convection loss ($M - E \pm R \pm S$) against the cooling effect represented by $\sqrt{V}\ (T_S - T_A)$.

The data represent four series of experiments, conducted, respectively, with warm-walls, neutral walls (equal to air), cool walls, and cold walls. For each series the interval between wall and air temperature was the same throughout, with varying air temperature, and, hence, varying calorie demand. As in figure 1, figure 2 shows that where the environ-

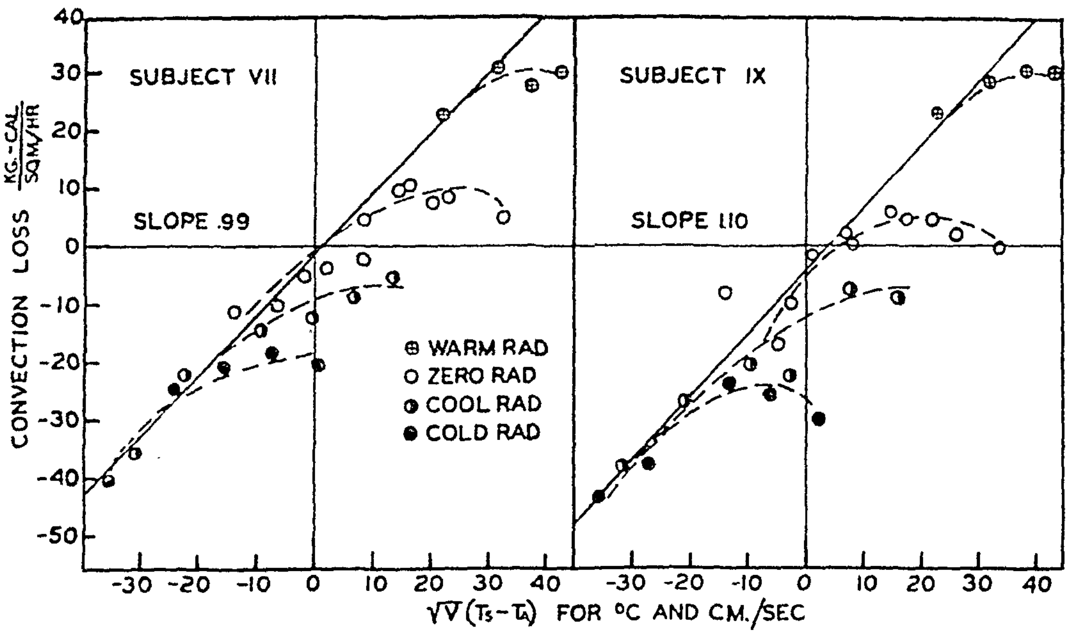

Fig. 2. Graph of convection loss plotted against product of air velocity and temperature differential for determination of convection constant.

ment is warm a common linear relationship is apparent and the line approximately intercepts the origin. From this line, we find that the K_C for unit air velocity is 0.99 for subject VII and 1.10 for subject IX.

In our earlier work with nude subjects we computed convection constants not per unit air movement but for standard air movement of eight centimeters per second. The values obtained (Gagge, Winslow and Herrington, 1938), when converted to unit area of subject and to unit air velocity are somewhat higher than those here reported. We have reason to believe—as will be indicated in a later paragraph—that the air velocities must have often exceeded the assumed value and the figures presented are, therefore, too high on a unit air velocity basis. Working with the present subjects (VII and IX), lightly clothed and with careful check on air move-

ment, we have reported a value of 1.04 per square meter of body surface as the basic convection constant, to be multiplied by the square root of air velocity to yield the total convection constant (Winslow, Gagge and Herrington, (1939). We believe that for both nude and clothed subjects in a semi-reclining posture the value of this basic constant is close to unity.

Storage values and skin temperature changes. It is obvious from the deviation of cold points in our curves from the straight lines of figures 1 and 2 that our estimates of storage for cold environments on the assumptions made above are much in error. We are confirmed, therefore, in our previous conviction that the only sound basis for estimating storage (outside the zone of linearity) is by difference—after accurate determinations of radiation and convection.

In the method of estimating storage from changes in skin and rectal temperatures, there are two possible pitfalls. In the first place, the question arises, from what point one should measure the rate of fall in these two temperatures. In the preliminary computations from which figures 1 and 2 were prepared, we took the mean rate of fall for successive 40-minute periods (40–80 min., 80–120 min., 120–160 min., and 160–200 min.). For the purpose of determining linearity relationships this—or any other rational assumption—would serve equally well. For determining absolute values, however, it seems erroneous to consider only the fall after the first 40 minutes, since, as we have shown (Winslow, Gagge and Herrington, 1939), the skin temperature falls very rapidly on initial exposure to a cold condition, as a result of immediate vasoconstriction. Storage, on the other hand, must be a much slower process, only catching up gradually with the primary physiological reaction. It seems, therefore, unsound to compare storage with the fall in skin temperature which occurs subsequent to the initial vasoconstriction.

In the second place, the computation of storage from changes in T_S and T_R involves a purely arbitrary assumption of the ratio to be assigned to each. It is obvious that this ratio must vary widely with the conditions of the experiment. In a slightly cool environment, only the very superficial areas are chilled; while under very cold conditions the area of tissue chilling must be deeper. It seems highly possible that in extreme circumstances, the bones and other less vascular parts of the body may be well below the temperature maintained in the blood stream which circulates so largely through organs such as the liver and brain where heat production is considerable.

From the results in table 1, we can make some estimate of what the actual significance of skin and rectal temperatures may be with respect to real storage. It will be noted that this table contains, in addition to columns for air temperature and wall temperature, a column for operative temperature. The figures in this column represent the combined cooling

effect of air and walls, as determined by the appropriate weighting of the physical effects of radiation and of convection, with the subjects concerned

TABLE 1*

Mean responses of two unclothed subjects to variations in air and wall temperature

CONDITION	T_A	V	T_W	T_O	T_S	T_R	M	E	R_H	C	CAL. S	Obs. S	K
	°C.	cm./sec.	°C.	°C.	°C.	°C.		kg. cal. per square meter per hour					kg.-cal./sq.m.-hr.-°C.
4	26.9	7.2	40.0	34.9	35.3	37.14	45.5	−42.2	20.4	−23.2	−0.6	−0.5	24.4
8	23.6	7.7	36.0	30.9	35.0	37.11	46.7	−21.2	4.3	−32.8	0	3.0	23.5
12	19.5	7.7	31.5	26.3	33.1	36.82	46.0	−15.6	−6.6	−39.0	2.2	15.2	16.4
16	15.9	7.6	28.4	23.2	31.7	36.51	47.3	−13.7	−13.3	−44.8	6.5	24.5	14.9
21	41.4	7.3	40.7	41.2	36.1	37.31	44.4	−70.9	20.1	14.8	−1.9	−8.4	29.8
31	38.5	5.3	38.1	38.1	35.9	37.34	45.8	−66.7	8.8	6.3	−1.9	+5.8	35.8
17	36.5	7.4	35.7	35.9	35.7	37.32	45.4	−52.1	0	2.3	−1.2	4.4	30.7
29	34.5	5.7	33.9	34.2	35.4	37.17	45.6	−44.1	−6.3	−2.1	−1.2	6.9	29.6
1	32.8	7.6	32.8	31.2	35.6	37.32	46.1	−29.4	−11.8	−8.1	−0.9	13.2	34.4
25	31.7	6.4	30.6	31.1	35.1	37.11	45.1	−23.4	−18.6	−8.9	−0.9	5.8	25.2
5	28.6	7.9	28.9	28.6	34.6	37.03	44.8	−12.7	−23.4	−17.4	0	8.7	22.0
35	27.5	5.6	26.7	27.4	33.9	36.98	47.6	−12.8	−29.0	−15.6	+1.5	9.8	18.6
9	25.0	8.6	24.9	24.6	33.6	36.78	44.2	−10.3	−36.0	−26.0	+3.1	28.1	22.7
39	23.8	6.7	22.9	23.7	32.2	36.91	48.7	−11.8	−36.5	−22.0	+3.4	21.6	14.9
13	21.7	10.8	21.3	20.0	31.9	36.81	45.1	−10.1	−40.1	−41.3	+5.6	46.4	18.5
23	47.8	6.8	35.6	40.4	35.9	37.34	47.0	−79.8	−1.3	31.9	−1.5	2.2	34.1
19	43.4	7.1	31.4	36.2	35.2	37.15	46.5	−53.8	−15.8	22.5	−1.5	0.6	24.2
3	38.9	7.6	28.0	32.4	35.5	37.25	44.4	−30.5	−30.7	9.7	−1.2	7.1	29.4
7	34.7	7.6	24.4	28.7	34.5	36.96	42.7	−18.8	−40.4	.5	0	16.0	23.8
11	31.2	8.4	21.2	25.5	33.7	37.01	46.6	−11.4	−49.0	−7.5	3.7	21.3	20.5
15	26.9	8.3	18.0	22.0	32.1	36.75	46.2	−9.8	−54.0	−15.4	6.5	33.0	17.0
18	48.7	7.0	26.9	35.7	35.2	37.08	46.0	−50.8	−33.7	37.1	0	1.4	25.2
2	44.7	7.6	24.0	32.6	35.3	37.33	43.8	−31.1	−45.3	26.9	−1.2	5.7	24.4
6	39.6	7.6	20.6	28.8	34.3	36.95	43.8	−14.3	−53.6	15.2	+0.9	7.9	19.5
10	35.4	8.7	17.8	25.7	33.3	37.00	44.2	−13.7	−59.7	6.5	+3.7	21.7	17.8
14	31.4	8.1	14.0	21.9	32.0	36.67	45.9	−14.2	−67.5	−1.8	+5.9	37.6	20.0

* T_A, ambient air temperature; V, air movement measured in booth with chair but without subject; T_W, mean radiant wall temperature; T_O, operative temperature including the combined effect of wall temperature, air temperature, and air movement; T_S, mean skin temperature; T_R, rectal temperature; M, the metabolism; E, evaporation loss; R, the radiation exchange; C, the convection exchange; Cal. S, storage calculated from change in skin and rectal temperature weighted 1:2, respectively; Obs.S, the algebraic sum of $M - E \pm R \pm C$; K, conductance, the ratio of $M + S$ to $T_R - T_S$.

and the wall temperature, air temperature and air movement which obtained. It is the temperature of air which—with equal wall and air

temperature and air velocity of 7 cm. per second—would exert the cooling effect actually produced. The table contains two columns for storage, one computed by weighting change of skin temperature and rectal temperature in the respective ratios of 1 and 2, the other as determined by the algebraic sum of $M - E \pm C \pm R$. It will be noted that at all operative temperatures below 40°C., true storage is higher than storage estimated from ΔT and that below an operative temperature of 26°C., the difference becomes very great, rising from 10 up to over 40 kilogram calories.

To test further this somewhat surprising result—and to determine whether any different ratio between skin and rectal temperatures would better describe storage phenomena—we conducted two special experiments extending over longer periods of time and with more frequent successive observations throughout the period involved.

In both these experiments, the air temperature was maintained as closely as possible at the skin temperature of the subjects, so that convection loss ranged only from −3 to +4 kilogram calories per square meter per hour. The actual air temperature varied from 31.9° to 35.3°C. In experiment A, the wall temperatures started at 37.1°C. and were kept between that point and 36.9°C. for 100 minutes. The wall temperature was then rapidly reduced to 25.8°C. at 140 minutes, 19.9° at 160 minutes, and 18.2° at 180 minutes. It was then maintained between 17.1° and 17.8° until the close of the experiment at 360 minutes. Thus, the operative temperature averaged 36.1° during the first 120 minutes and 24.6° during the last 200 minutes.

In experiment B, the walls were cold throughout, varying between 14.3° and 15.5°C., a condition maintained for 360 minutes. The mean operative temperature in this experiment was 22.9°C.

Figure 3 shows the general results obtained in these two experiments. Each point on the curve represents a 20-minute interval. The curves have been plotted arbitrarily as overlapping, although the two experiments were conducted on different days; and the point at which the walls were cooled in experiment A is indicated by a vertical line.

It will be noted that a slight radiation gain occurred before this point in experiment A, this gain plus the metabolism being balanced by high evaporative heat loss. After this point in experiment A, and throughout experiment B, there was a large loss of heat by radiation to cold walls. Convection interchange was negligible throughout. Metabolism fell at the beginning of each experiment (as is usual) and, later, rose slightly in experiment A and much more definitely in experiment B.

During the hot phase of experiment A, skin temperature fell very slightly and rectal temperature rose. During the cold phase of this experiment, both skin and rectal temperature dropped, but both became stabilized during the last hour. During experiment B, both skin tempera-

ture and rectal temperature dropped; but again, both were essentially stabilized during the final hour.

In figure 4 we have presented regional temperatures for the fifteen points on the body surface studied. It will be noted that for the last hour of experiment A every region showed a stable or rising skin temperature

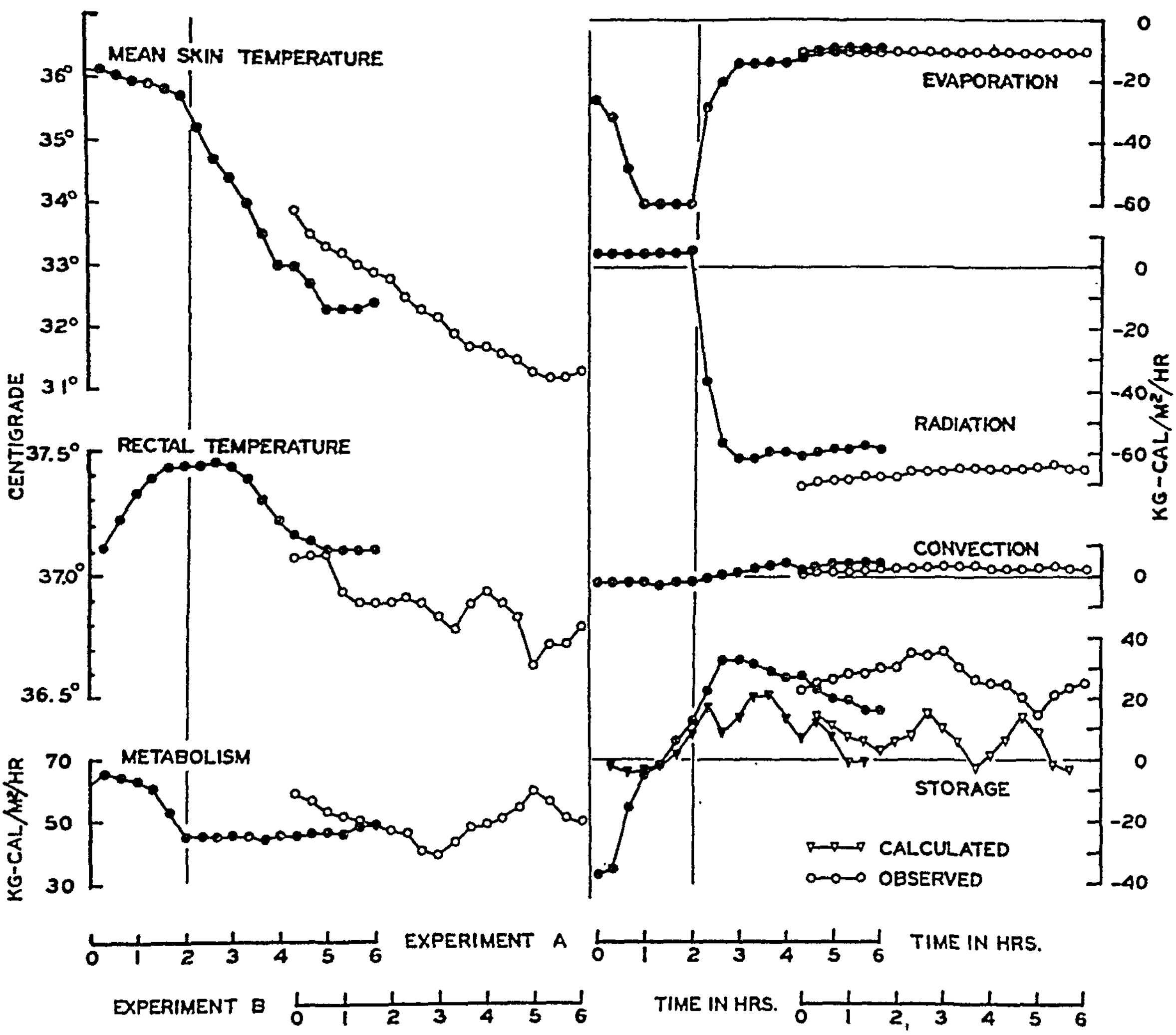

Fig. 3. Progressive temperature changes and changes in the factors of thermal balance on exposure to a cold environment.

Experiment A. Operative temperature of 36°C. for the first 2 hours, operative temperature of 25°C. for the last 4 hours.

Experiment B. Operative temperature of 23°C. for the entire 6 hours.

(for the last three 20-min. periods) except the chest, the back of the calf, and the seat. In experiment B, local skin temperatures were stable or rising for over two hours in the case of the forehead, scalp, fore-arm, shoulder, side-calf, back-calf, and seat; for an hour or more in the case of the cheek, upper arm, hand, chest, trunk, kidney, and thigh; only the instep failed to stabilize its temperature.

During the whole last two hours of this experiment, the rectal temperature fell only from 36.9° to 36.8°; and the mean skin temperature fell only from 31.7° to 31.3°C. Yet during this period the body was producing

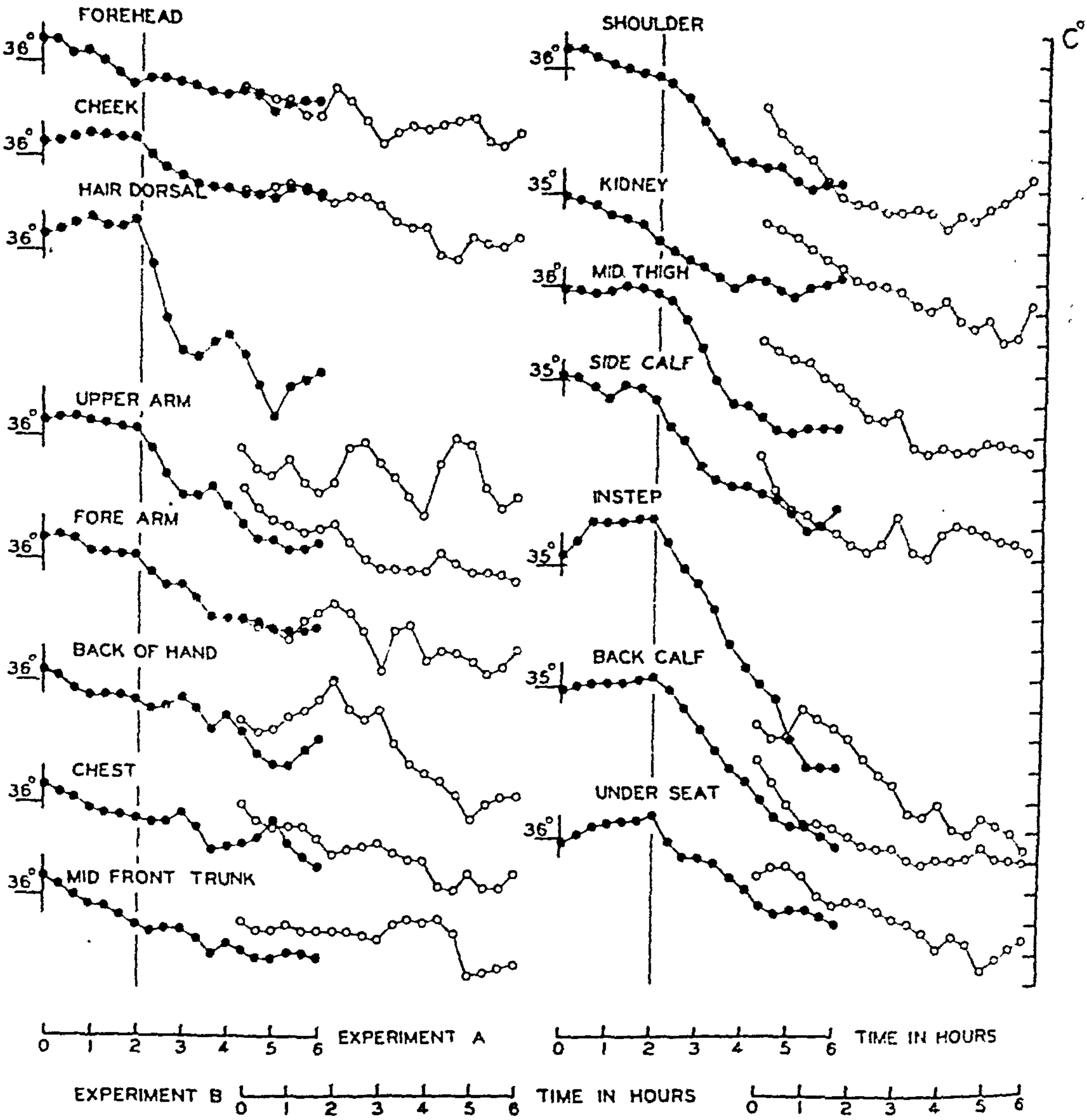

Fig. 4. Progressive changes in temperatures of fifteen regions on the body surface on exposure to a cold environment.

Experiment A. Operative temperature of 36°C. for the first 2 hours, operative temperature of 25°C. for the last 4 hours.

Experiment B. Operative temperature of 23°C. for the entire 6 hours.

53 kilogram-calories of heat per square meter of body surface per hour by metabolism, gaining 2 kilogram-calories by convection, losing 11 kilogram-calories by evaporation, and 65 by radiation—leaving 21 kilogram-calories which must have been stored by cooling of body tissues. The graph at

the bottom of figure 3 shows the storage throughout the experiments as computed in this way by difference and (triangles) the storage as computed from changes in skin temperature and rectal temperature weighted respectively in the ratio of 1:2.

It seems clear from this graph that the 1:2 ratio gives no adequate picture of storage. Furthermore, from the data cited above, as to stable skin and rectal temperatures maintained for considerable periods while the body was actually cooling off rapidly, it seems clear that no reasonable weighting system can be used which will represent what happens when the body is subjected to severe chilling. With moderate atmospheric conditions the 1:2 ratio may be useful (Winslow, Herrington and Gagge, 1939). Under more extreme conditions, however (both on the hot and the cold side), we are forced to conclude that the vaso-motor system has an extraordinary power of stabilizing both rectal and skin temperature while considerable chilling of less vascular tissues is going on. This cannot, obviously, proceed for an indefinite period; and toward the end of experiment B we have evidence that a compensatory increase in metabolism has begun. It may well be that the stimulus to this increased metabolism arises from the chilling of the muscular tissues themselves; independent of the relatively stable temperatures of the blood stream.

It may be of interest to note that, during the hot phase of experiment A, the difference between rectal and skin temperature rose from 1° to 1.7°C. and the conductance rose from 24 to 40 and fell again to 33. During the cold phase, the $T_R - T_S$ differential rose to 4.8° and the conductance value fell to 13—both these values remaining constant for the final hour. In experiment B, the $T_R - T_S$ differential rose from 3.2° to 5.5°C. and the conductance fell from 26 to between 13 and 14. Again, these values were constant for the final hour.

Specific influence of walls and air. After this essential discussion of basic fundamentals, we may now turn to the major objective of the present study, the differential influence of wall temperatures above and below air temperatures.

Our basic data are presented in table 1, each horizontal line representing the average of two experiments on each of the two subjects studied. Four sets of experiments were made with walls about 12°C. warmer than the air, eleven sets with air and walls equal, six sets with walls 10° to 12° cooler than the air, and five sets with walls 16° to 20° colder than the air.

The results are presented graphically in figure 5 for the average data for the period from 40 to 200 minutes.

The data for radiation and convection heat interchanges (in the upper right of the chart) show how successfully these factors were varied over a range of some 50 kilogram-calories per square meter of body surface for the same operative temperature. The graphs for metabolism and evapo-

ration show essentially identical results for all four experimental conditions.

Skin and rectal temperatures are the same at a given operative temperature for the equal and cool and cold wall conditions. Both these tempera-

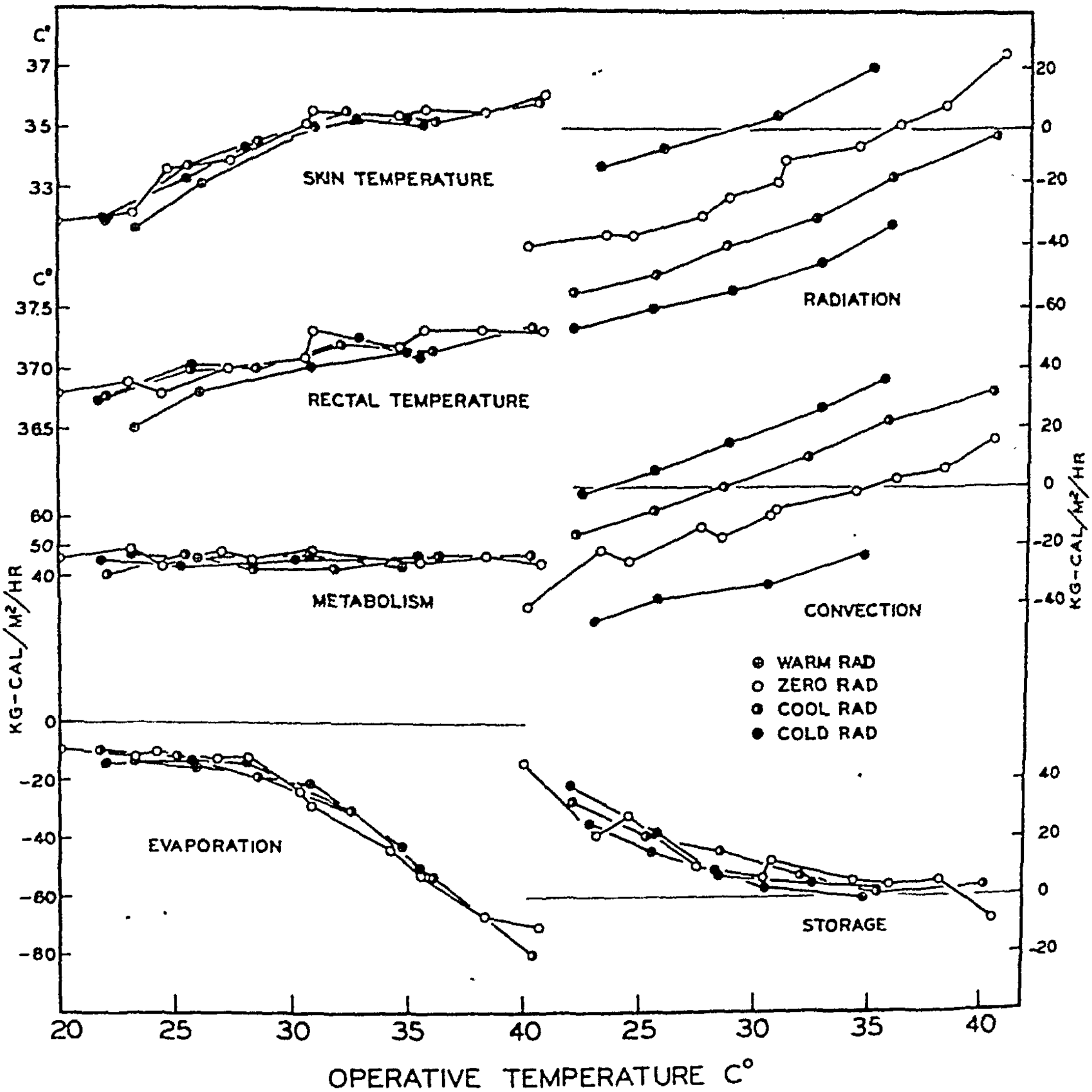

Fig. 5. Temperature values and values for various factors in thermal balance plotted against operative temperature with mean radiant temperatures above, equal to, and below air temperatures.

tures are slightly lower, at a given operative temperature, with the cold-air warm-wall situation and storage for this experimental condition is very slightly reduced as a result of this lower skin temperature.

These results, then, confirm in a qualitative sense those reported in an earlier communication (Herrington, Winslow and Gagge, 1937). The re-

duction in skin temperature associated with the cold-air warm-wall situation is, however, much less (0.5°–1.0°) than in our earlier studies (1°–2°). In the previous study, a constant rate of air movement was assumed. In the present studies air movement was recorded for each experiment; and it may be seen from table 1 that at an operative temperature of 40° air velocity was between 6 and 7 cm. per second, while at 30° the corresponding value was about 8 cm., and at 20° nearly 11 cm. If this occurred in our new booth, the phenomenon must have been much more marked in the smaller, old booth used in 1937. We are inclined, therefore, to believe that the earlier results were influenced by increased air movement at low operative temperatures. We may conclude from our present studies that the cold-air warm-wall situation does exert a cooling influence greater than would be predicted from its operative temperature, but that the difference is slight. It is evident that the influence exerted has nothing to do with specific effects of radiation and convection since a hot-air cold-wall situation (even with a 20° differential) gives results identical with those of equal wall and air. The phenomenon must be due to an influence of cold air alone. The magnitude of the effect is such that it can easily be explained, as we have suggested, in a study on clothed subjects (Winslow, Herrington and Gagge, 1938) "by local chilling of the membranes of the upper respiratory tract (which might be far more effective in stimulating vaso-constriction than would be expected from its direct effect on caloric loss); by local chilling of the body by the metal supports of the chair in which the subject reclines (although the seat of the chair is cloth); and by temporary increases in convection loss when the subject enters the room or moves his arms or body."

CONCLUSIONS

1. A new experimental booth is described, which provides more accurate control of the various experimental variables in heat interchange than was previously possible.

2. Radiation areas as determined in this new booth are found to lie between 70 and 75 per cent of the DuBois area.

3. Convection constants are found to lie close to a value of 1.0 multiplied by the square root of air velocity in centimeters per second.

4. It is shown that, when the body is subjected to markedly cold or hot conditions, storage can not be estimated from changes in skin temperature and rectal temperature. Both these temperatures may be maintained by the vaso-motor system at a stable level for considerable periods, in spite of the fact that appreciable heating or chilling of less vascular tissues is taking place. Storage, under such conditions, can be measured most adequately by difference—from the algebraic sum of metabolic heat production, evaporative heat loss and gain, or loss by radiation and convection.

5. In general, the influence of air and walls is accurately represented by the fundamental concept of operative temperature as previously advanced by us. So far as cold-wall hot-air situations and equal wall and air situations are concerned, physiological responses exactly follow operative temperature (as computed from mean radiant temperature and radiation area combined with air temperature, air movement, and convection constant). With a cold-air hot-wall situation, however, skin and rectal temperatures are somewhat lower—for a given operative temperature—than with equal wall and air or with hot-air and cold walls. The difference is slight (0.5°–1.0°) and can be accounted for by the cooling effect of the cold air upon the membranes of the nose and throat, and, perhaps, also by additional heat loss to the metal supports of the subject's chair and to increased convective heat loss due to movements of the arms and legs.

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STANDARD OPERATIVE TEMPERATURE, A GENERALIZED TEMPERATURE SCALE, APPLICABLE TO DIRECT AND PARTITIONAL CALORIMETRY

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In view of the trend[1] in recent years among workers in the general field of metabolism and calorimetry to emphasize the physiology of heat elimination from the animal body, and since such processes of heat elimination are so fundamentally governed by the physical properties of the environment itself, a generalized simplification and standardization of these properties seems in order. It is the purpose of the present paper to define and derive a generalized environmental temperature scale, that combines as a single measurement certain of the thermal effects of the physical environment, aqueous or atmospheric, and in the latter case for any combination of radiant temperature, ambient air temperature and air movement. Use of such a generalized temperature scale as an independent variable would give to experimenters working under diverse conditions a common reference measurement—and one which permits differentiation between effects of a purely physical nature and those of a physiological nature.

DEFINITION AND DERIVATION. The formal basis of standard operative temperature, T_O, is Newton's Law of Cooling and this factor is defined as the equivalent environmental temperature with which a warm body (such as a human or animal subject) with surface temperature, T_S, exchanges heat at a *standard* cooling rate, K_O. From Newton's Law

$$\text{Heat Loss to Environment} = K_O\,(T_S - T_O), \tag{1}$$

our definition may be written

$$T_O = T_S - (\text{Heat Loss to Environment})/K_O. \tag{2}$$

The above definition (2) is universally applicable to all types of Calorimetry, where the Calorie loss (aside from evaporative cooling) from the animal subject is measured without particular attention to its mode of elimination. By environment one means the physical complex actually

[1] Recent comprehensive reviews in the literature indicating this trend are given by Deighton (1933), Kleiber (1937), Murlin (1938), Burton (1939) and Dill (1939).

surrounding the animal; it may be anything from a water bath to a respiration chamber.

In Partitional Calorimetry (Winslow, Herrington and Gagge, 1936), where the mode of heat loss to the environment is of primary interest, the factors, mean radiant wall temperature (T_W), ambient air temperature (T_A) and air movement, V, must be considered. Relative humidity is omitted from the present discussion as it affects only the efficiency of evaporative cooling, a regulative process separately measurable and distinct from the direct heat loss to the environment by radiation and convection.

For the present let us consider an environment where air movement is constant; under this condition loss by radiation and convection for the animal subject may be expressed as

$$R + C = (A_R/A_D)\, k_r\, (T_S^4 - T_W^4) + K_C(T_S - T_A), \tag{3}$$

where temperatures are absolute in the 4th power radiation Law, k_r and K_C are the universal radiation constant (4.92×10^{-8} kg.cal./m.²/hr./°C.) and total convection constant respectively, and A_R/A_D is the ratio of the radiation area of the animal subject to its total area. As a first approximation (3) may be rewritten

$$R + C = K_R\,(T_S - T_W) + K_C\,(T_S - T_A), \tag{4}$$

which equation on substitution in our definition (1) or (2) gives

$$T_O = \frac{K_R T_W + K_C T_A}{K_R + K_C}, \tag{5}$$

and

$$K_O = K_R + K_C. \tag{6}$$

From (5) Operative Temperature is actually the average of wall and air temperatures weighted according to their relative effectiveness as described by the radiation and convection constants. This special application of operative temperature was first used in Partitional Calorimetry (Winslow, Herrington and Gagge, 1937a; Herrington, Winslow and Gagge, 1937) to combine as a single variable the temperature equivalents of the radiative and convective environments.

It has now been shown experimentally (Winslow, Gagge and Herrington, 1939) that the loss by convection is proportional to the square root of the air movement for the human body in a semi-reclining position up to air movements of 250 cm. per second. It is reasonable to assume that this relationship is generally true for any body size or position within the above limits of air movement, a limit considerably higher than normal under indoor conditions. Let us ask at what temperature, T_A', at standard air

movement, V_O, does one lose the same heat by convection as at the observed temperature, T_A, and air movement, V. From the equation for convection loss

$$k_c\sqrt{V}(T_S - T_A) = k_c\sqrt{V_O}(T_S - T'_A),$$

we have

$$T'_A = \sqrt{V/V_O}\, T_A - (\sqrt{V/V_O} - 1)\, T_S. \tag{7}$$

The presence of T_S in (7) is necessary since T_A' is equal to T_A unless the surface temperature of the subject is different from the air temperature itself.

If we now assume that (5) is valid for a standard air movement, V_O, by substituting (7) in (5) we have the operative temperature in terms of T_W, T_A, and V.

$$T_O = \frac{K_R}{K_O}[T_W] + \frac{K_C}{K_O}[(\sqrt{V/V_O})\, T_A - (\sqrt{V/V_O} - 1)\, T_S], \tag{8}$$

where the standard cooling rate, K_O, is equal to

$$K_R + k_c\sqrt{V_O}. \tag{9}$$

(8) describes the equivalent temperature in which a subject, with the observed surface temperature, T_S, would lose the same amount of heat at a standard cooling rate, K_O, as by radiation and convection in the original environment.

By standardizing K_O and its associated air movement, V_O, a temperature scale is established on a sound calorimetric basis, if one measures first the surface temperature of the animal subject and, secondly, either the total heat loss to the environment by direct calorimetry or the individual factors, wall temperature, air temperature, and air movement, themselves.

APPLICATION. Application of the methods described above will be limited for the sake of brevity to two experimental cases with the human body; 1, comparison of certain data taken in a Water Bath Calorimeter (Burton and Bazett, 1936) with those of the Russell Sage Calorimeter (Hardy and DuBois, 1937), and 2, comparison of unpublished data from this laboratory taken in the Partitional Calorimeter for various degrees of air movement when air and wall temperature are equal. The third possibility, the condition in which wall and air temperature independently vary has been already described in the literature (Herrington, Winslow and Gagge, 1937; Winslow, Herrington and Gagge, 1938; and Winslow, Gagge and Herrington, 1940).

Case 1. In both the Water Bath Calorimeter and the Russell Sage Calorimeter an evaluation of skin temperature is possible. In the former, since the bath is extremely well stirred, the bath temperature is practically

equal to the skin temperature, while in the latter a direct measurement is possible. For both, the total caloric loss by the body to the environment is measured directly. In each case the requirements necessary to derive standard operative temperature as originally defined by (2) are satisfied.

In table 1 are presented data derived from those obtained for a subject in the Water Bath Calorimeter by Burton and Bazett. The value of operative temperature has been calculated from equation (2), using a factor 5.3 kgm.cal./m.^{2}/hr./°C., for K_O, a value equal to the cooling constant

TABLE 1

*Calculation of operative temperature for data on subject H C B in water bath calorimeter**

CONDUCTANCE	HEAT LOSS TO BATH	BATH TEMP.	HEAT LOSS ÷ 5.3	T_O	CONDUCTANCE	HEAT LOSS TO BATH	BATH TEMP.	HEAT LOSS ÷ 5.3	T_O
kg. cal. / sq. m./hr./°C.	*kg. cal. / sq. m./hr./°C.*	*°C.*	*°C.*	*°C.*	*kg. cal. / sq. m./hr./°C.*	*kg. cal. / sq. m./hr./°C.*	*°C.*	*°C.*	*°C.*
17.7	27.6	35.35	5.21	30.14	29.3	25.8	35.52	4.87	30.65
9.8	44.7	32.05	8.43	23.62	28.2	28.5	35.52	5.38	30.14
34.0	38.8	35.88	7.32	28.56	28.8	29.1	35.52	5.49	30.03
28.5	33.0	35.88	6.23	29.65	7.6	33.5	32.26	6.32	25.94
11.9	66.3	31.26	12.51	18.75	7.6	32.0	32.26	6.04	26.22
10.6	57.1	31.26	10.77	20.49	7.9	31.8	32.26	6.00	26.26
36.6	25.2	35.92	4.76	31.16	22.5	19.6	36.26	3.70	32.56
9.5	32.1	33.12	6.06	27.06	21.4	22.7	36.26	4.28	31.98
8.7	28.1	33.12	5.30	27.82	15.9	38.0	34.34	7.17	27.17
7.9	24.8	33.12	4.68	28.44	15.2	34.0	34.34	6.42	27.92
10.9	30.6	33.72	5.77	27.95	10.9	24.1	34.34	4.55	29.79
10.9	30.1	33.72	5.68	28.04	10.9	32.0	33.62	6.04	27.58
11.9	32.5	33.72	6.13	27.59	10.9	31.2	33.62	5.89	27.73
10.1	27.1	33.72	5.11	28.61	10.1	35.1	33.01	6.62	26.39
17.0	107.2	30.22	20.20	10.02	6.7	22.5	33.00	4.25	28.75
11.9	71.6	30.27	13.51	16.76	10.6	28.6	33.57	5.40	28.17
11.9	69.7	30.28	13.15	17.13	11.3	30.6	33.57	5.77	27.80
11.9	68.5	30.28	12.93	17.35	22.6	29.2	35.03	5.51	29.52
11.7	66.5	30.29	12.55	17.74	21.2	28.8	35.03	5.43	29.60

* Note: the author is indebted to Doctor Burton and Doctor Bazett for the use of the original data from their publication (1936) in preparation of the above table.

of the Russell Sage Calorimeter. In figure 1 the physiological variable, conductance (Burton and Bazett, 1936; Winslow, Herrington and Gagge, 1937a), a measure roughly proportional to the peripheral circulation of the skin, is plotted against operative temperature for a subject in each calorimeter. The data for the Russell Sage subject are taken directly from the literature (Hardy and DuBois, 1937) to which the reader is referred. Thus, figure 1 compares the vascular response of two subjects in widely different types of calorimeters on a common operative temperature scale (in this particular case the temperature of the Russell Sage

Calorimeter is also the operative temperature). The following conclusions may be reached from the figure.

a. Vascular regulation or, rather, vasodilatation begins to increase approximately at the same operative temperature in spite of the fact that

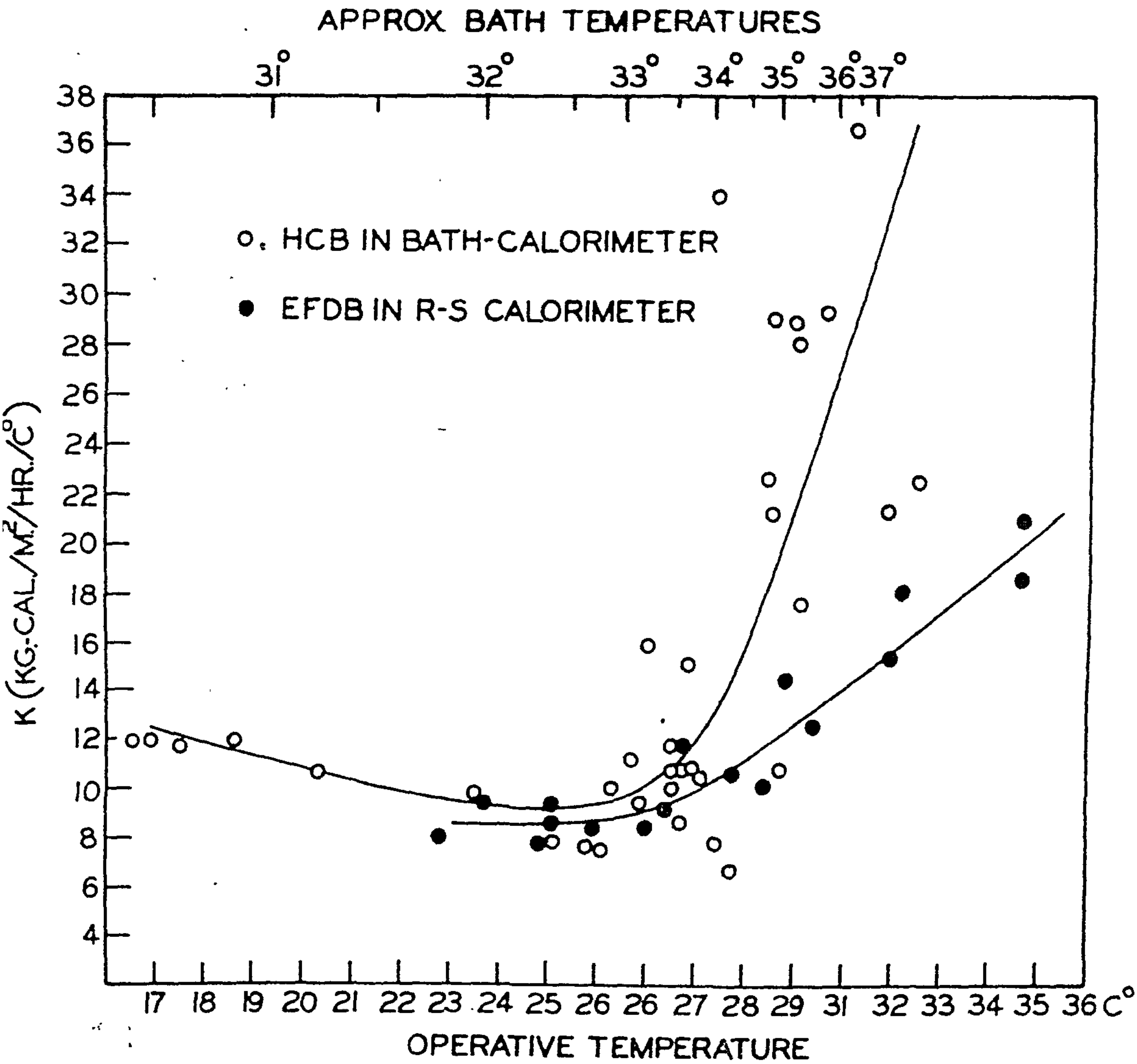

Fig. 1. The relation between conductance (a measure of peripheral blood flow) and operative temperature for a subject (EFDB) in the DuBois-Hardy Calorimeter, and another (HCB) in the Burton-Bazett Water Bath Calorimeter.

the media of heat loss are quite different. This fact suggests that vascular regulation in the two instances results from the same thermal stimulus.

b. The large difference in the two cases between the rate of rise in peripheral circulation with increasing operative temperature indicates the relative economy in vascular effort provided by the process of evaporative

regulation which, while present in the Russell Sage Calorimeter, is absent in a water bath.

Case 2. A series of data averaged from those taken on two male subjects in a semi-reclining position under conditions of equal wall and air but under various air movements is presented in table 2. In the first three columns are presented the basic physiological data, metabolism (M), evaporation loss (E), and skin temperature (T_S). In the next two columns the air temperature (equal to that of the wall) and air movement are recorded. In the sixth column the equivalent air temperature for a standard air movement of 7.6 cm. per second is calculated from equation (7).

TABLE 2

Calculation of operative temperature (V_O = 7.6) for data where air movement varies

CONDUCTANCE	M	E	T_S	$T_A = T_W$	V	T'_A	T_O
kg.-cal./sq. m.-hr-°C.	kg.-cal./sq. m.-hr.	kg.-cal./sq. m.-hr.	°C.	°C.	cm./sec.	°C.	°C.
1	52	49	35.9	35.3	7.6	35.3	35.3
2	46	44	35.1	35.1	51	35.1	35.1
3	48	42	35.1	35.3	132	35.9	35.6
4	49	44	35.2	35.3	264	35.8	35.6
5	47	30	35.5	33.1	7.6	33.1	33.1
6	48	21	35.5	33.4	51	30.1	31.8
7	50	16	35.3	33.7	132	28.6	31.2
8	45	16	35.2	33.9	264	27.5	30.7
9	46	21	34.9	31.3	7.6	31.3	31.3
10	46	16	33.8	31.4	51	27.6	29.5
11	47	13	33.0	31.4	132	26.3	28.9
12	50	14	33.1	31.4	264	23.1	27.3
13	43	13	34.1	27.3	7.6	27.3	27.3
14	50	12	32.1	27.6	51	20.4	24.0
15	49	12	31.3	27.7	132	16.3	22.0
17	52	12	32.6	22.4	7.6	22.4	22.4
18	54	12	31.2	22.3	51	8.1	12.5

The data in table 2 were taken in a small partitional calorimeter where a value of 0.5 is found for each of the ratios K_R/K_O and K_C/K_O, when V_O equal 7.6 cm. per second. In the final column of the table the standard operative temperature is calculated from equation (6). The basic physiological data are plotted against operative temperature in figure 2, from which the following conclusions may be reached.

a. Metabolism is approximately constant within the temperature range studied.

b. After the data have been "normalized" by operative temperature, evaporative regulation begins at 31°C., this fact being true for all degrees of air movement. As evaporative regulation is a very sensitive index of any

change in heat balance with the environment, it is specially significant that this measure is completely "normalized" by operative temperature.

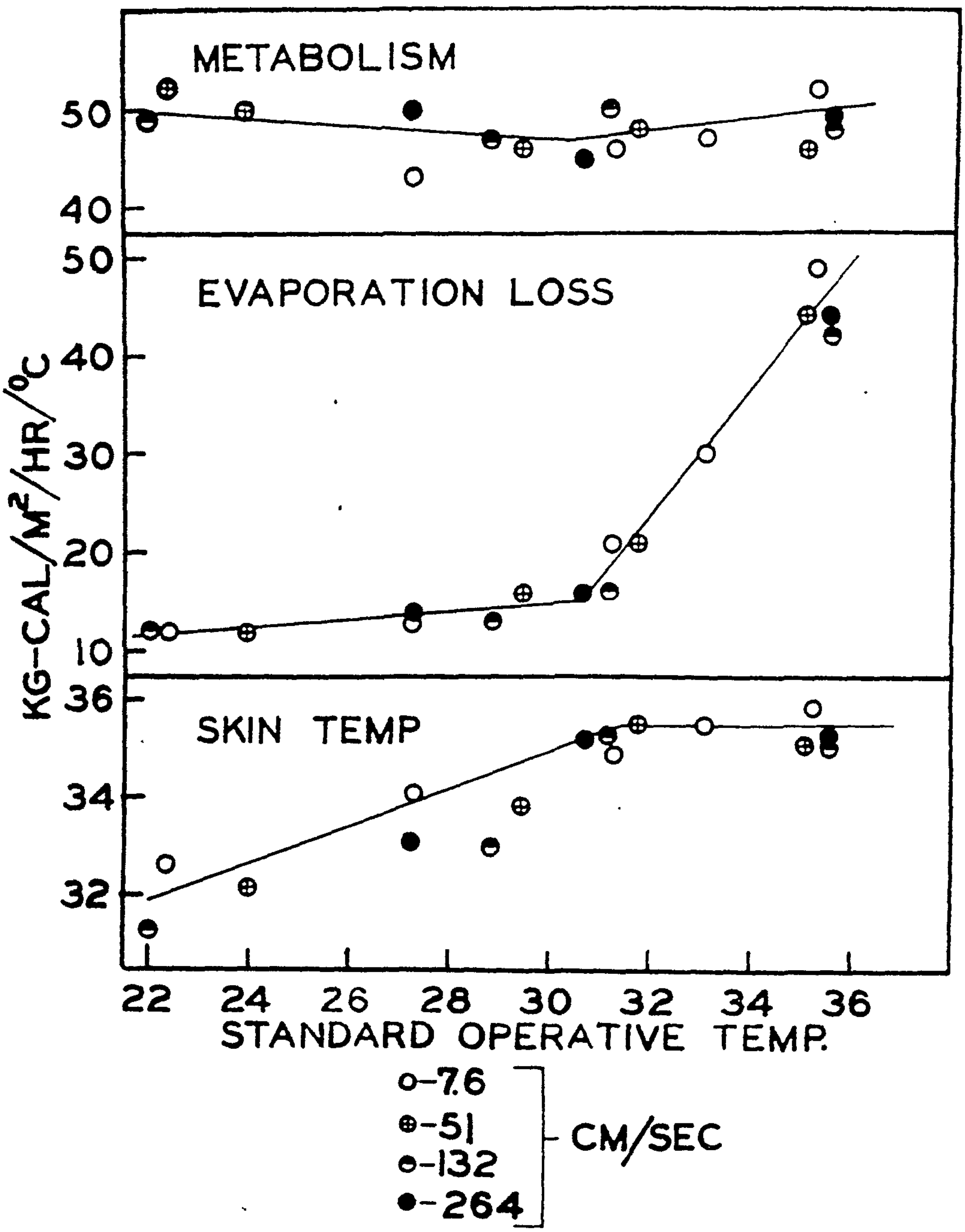

Fig. 2. The relation between metabolism, evaporation loss and skin temperature and operative temperature for various air movements and dry bulb temperatures in the Partitional Calorimeter.

c. The skin temperature curves also inflect at the critical point of 31°C. operative temperature, further indicating normalization.

d. The fact that, at skin temperatures below 31°C., points for high air movement but for the same operative temperature lie below the graph may indicate a vascular response to air movement influenced by other than thermal factors.

Standard K_O. In the original presentation of the two experimental cases discussed in the previous paragraphs no effort was made to generalize the use of operative temperature. In the first case, two different experimental methods of calorimetry were compared on a temperature scale derived from one of them. In the second case, a family of experiments similar in method but under different conditions, were reduced to a common independent variable. The real value of the concept of operative temperature should lie in its ability to reduce all methods of calorimetry to a common single independent variable. This is apparently possible by defining and standardizing on a value of the cooling constant, K_O.

Let us define the standard value for K_O for a given animal subject to be the value of its Newton Cooling constant when it is placed under basal conditions in an ideal environment, where the wall and air temperatures are equal, where air movement is unity, where heat production exactly balances heat loss to the environment, and where neither evaporative nor vascular regulation are necessary. For the human body in thermal equilibrium

$$K_O = \frac{\text{Basal metabolism less basal evaporation}}{\text{Difference between skin and air-wall temperature}}.$$

According to our definition, if we assume a value of 40 kgm.cal./M.^{2}/hr. for the basal metabolism, 10 kgm.cal./M^2/hr. for the basal evaporation, and 35°C. for the optimum value for the skin temperature, we have

$$K_O = \frac{30}{35° - T_A}. \tag{10}$$

From equation (9) above it follows for unit air movement that

$$K_O = K_R + k_c. \tag{11}$$

K_R, strictly speaking, is not a constant since the true radiation law is a fourth power difference, not a linear difference. A more exact expression for (11) is

$$K_O = \left[4k \times (A_R/A_D) \times \frac{T_W^3 + T_S^3}{2}\right] + [k_c], \tag{12}$$

whose terms have already been defined above.

Recent experiments (Winslow, Gagge and Herrington, 1940) have shown that the most probable value for A_R/A_D is 75 per cent and the value for

k_c is 1.0 when V is expressed in centimeters per second. In the light of our earlier substitutions (12) now becomes

$$K_O = 0.75 \times (4 \times 4.92 \times 10^{-8}) \times \left[\frac{T_W^3 + (273° + 35°)^3}{2}\right] + 1.0. \quad (13)$$

Both (10) and (13) are numerically satisfied simultaneously when K_O and T_W are equal to 5.2 and 29.2°, respectively. Therefore, in an ideal environment of 29.2°C. with unit air movement, the value of the standard cooling constant, K_O, is found to be 5.2 kgm.cal./m.²/hr. Under these same ideal conditions, K_R equals 4.2, and K_C equals 1.0.

The value for standard K_O of 5.2 kgm.cal./m.²/hr./°C. is extremely close to the cooling constant of 5.3 of the Russell Sage Calorimeter. This near equality, although fortuitous, is also fortunate as it gives added significance to the former value. Instead of referring operative temperature to a cooling rate under an ideal condition, one could give it more realistic meaning by referring to a cooling rate and a temperature, equivalent to that in a calorimeter of known and established physical properties.

In conclusion, a biophysical temperature scale for the human body generally applicable to all forms of calorimetry may be defined by either of the two relations

$$T_O = T_S - (\text{Heat Loss to the Environment})/5.2, \quad (14)$$

$$T_O = 0.81\,[T_W] + 0.19\,[\sqrt{V}\,T_A - (\sqrt{V} - 1)\,T_S], \quad (15)$$

where heat loss is expressed in kilogram-calories per square meter per hour and when V is measured in centimeters per second.

Discussion. The one physical property of the environment not included in operative temperature is relative humidity. Except under, perhaps, extremely warm saturated conditions in which no animal could long exist, the effect of humidity is of secondary importance on absorption of radiation and conductivity of the surrounding air and hence on the radiation and convection constants themselves. It is not believed necessary to include this effect in operative temperature. Humidity, however, does affect insensible perspiration and evaporative regulation. Its direct effect is to change the rates of secretion and evaporation, any change in which can only result in a change in mean skin temperature. However, as mean skin temperature is fundamental to our definition of operative temperature, operative temperature must include in its interpretation these secondary effects of humidity on the heat exchange by radiation and convection with the environment.

From partitional calorimetry studies (Gagge, Winslow and Herrington, 1938) on the human body it has been shown that, when evaporative regulation is completely successful, this loss increases by exactly the same amount as the calorie demand (loss by radiation and convection) of the environ-

ment decreases, and vice versa. The statement is also true for variations in humidity (Winslow, Herrington and Gagge, 1937b), as long as the evaporative rate is under 30 kilogram-calories per square meter per hour per centimeter of the Hg vapor pressure difference between skin and air—a value found to be the maximum effective cooling rate of the human body (Gagge, 1937). Because of this very exact correlation between evaporative regulation and calorie demand, and since calorie demand (or heat loss to the environment) is also fundamental to our definition of operative temperature, it may be generally stated that evaporation within the range of complete regulation is variant only with operative temperature and invariant with any of the individual physical factors of which it is composed.

The experimental validation of this latter conclusion for a subject at rest is found for T_O environments where wall and air temperature are independently varied (Winslow, Herrington and Gagge, 1937b), where air movement varies (see fig. 2 of this paper), and finally where humidity varies (Winslow, Herrington and Gagge, 1937a).

Operative temperature actually is a formal analytic expression, defined and derived from a well known physical principle. It is, strictly speaking, a *calorimetric* temperature. Until actually verified by experiment it implies no exact relation to the sensation of temperature or to the sensory equivalance in terms of hot or cold of the physical components of the environment. As such it is only a step to an end. The "Effective Temperature Scale" (Houghten and Yagloglou, 1923, 1924; Yaglou and Miller, 1925), sponsored by the American Society of Heating and Ventilating Engineers and currently used by many physiologists, is derived experimentally on a straight sensory basis. It combines in a single variable the sensory effect of not only air movement but also humidity. "Operative" and "Effective" temperatures must obviously have many properties and trends in common but the difference between the two scales is fundamental—the former is based on a known physical law, while the latter is derived from an experimental procedure where the sensations of temperature and comfort are the only discriminating factors defining the scale. From the latter one can make no accurate deduction in a calorimetric or thermodynamic sense nor are general physiological responses to it any more certain than to operative temperature. Somewhere between "Operative Temperature," analytically a pure calorimetric scale, and "Effective Temperature," experimentally a pure sensory scale, must lie a temperature scale to which all physiological responses, vascular, sensory, and metabolic are related—a truly physiological temperature scale.

From the preceding section it is quite apparent that operative temperature is essentially a biophysical quantity since skin temperature is included in its definition. It is a measure of the thermometric potential that governs the calorie exchange between the body and its surrounding en-

vironment, and at the same time it reduces this heat exchange to an equivalent temperature drop along a standard gradient from the body surface. By equilibration and standardization of the most complex environments all calorimetric procedures may be described in terms of a common reference scale, and with this scale it is possible to separate from any physiological temperature response its true physical component.

SUMMARY

Standard operative temperature based on Newton's Law of Cooling is defined as the equivalent environmental temperature with which a warm body (such as a human or animal subject) with surface temperature, T_S, exchanges heat at a *standard* cooling rate (excluding evaporation effects). Equations are derived from which one may calculate operative temperature when the skin temperature is measured and either the heat loss to the environment or the environmental factors wall temperature, air temperature, and air movement, are known. Operative temperature is a measure of the thermometric potential that governs the calorie exchange between the body and its surrounding environment. It also includes any humidity effect of the environment on this exchange. It is shown that the loss by evaporative regulation is variant only with operative temperature and invariant with any of the individual physical factors of which it is composed. Use of operative temperature is suggested as a standard reference scale for direct and partitional calorimetry and when one wishes to differentiate between effects of a purely physical nature and those of a physiological nature.

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FACTORS DETERMINING THE PRODUCTION OF VENTRICULAR FIBRILLATION BY DIRECT CURRENTS (WITH A NOTE ON CHRONAXIE)[1]

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In 1938, one of us (1) suggested that the sensitivity of the ventricle to fibrillating agents might perhaps be established quantatively by determining the strength of a brief shock which causes fibrillation when applied late in systole. Before such a criterion could be adopted, it was necessary to prove that such a localized brief stimulus causes fibrillation only when applied during this phase of the heart cycle; in other words, that the late portion of systole (ca 0.06 sec.) is a peculiarly vulnerable period. In a previous communication (2) we presented evidence that this is true for condenser as well as induction shocks. However, we soon found that the fibrillating action of somewhat longer D.C. and A.C. shocks is not limited to this period; indeed, under certain conditions, stimuli cause fibrillation when their effective portions fall entirely during diastole. It therefore became important to investigate the mechanisms of action of such D.C. and A.C. shocks in order to determine whether the fibrillating threshold to brief shocks is a valid general test for the sensitivity of the ventricle to fibrillating agents.

The present communication deals with the conditions under which fibrillation is produced *a*, by short rectilinear shocks which, temporally speaking, can fit into this period, and *b*, by direct currents of longer duration, which lap over the vulnerable phase. As regards the latter, experimental study has shown that our *a priori* conclusions regarding the stimulating action of such currents prove correct. However, experiment has brought into evidence additional factors which could not have been predicted by theoretical reasoning.

PROCEDURE. *Apparatus.* Numerous observations were made on 16 dogs. They were anesthetized, usually with morphine and sodium barbital; the chest was opened in the midline and the slit pericardium was

[1] This investigation was supported by a grant from the John and Mary R. Markle Foundation.

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stitched to the retracted chest opening. Thus, the heart was suspended in a pericardial cradle so that its position was influenced little by artificial inflation of the lungs. It may be noted parenthetically that, while such suspension of the heart is useful in precise stimulation of ventricular surfaces, the abnormal contacts between the heart, lungs and chest wall may modify the form of electrocardiograms recorded by standard leads. This, rather than abnormalities in the heart itself, accounts for some modifications in the configuration of our electrographic curves. However, these curves were always sufficiently distinctive to differentiate between normal and aberrant types of conduction.

Direct currents of various strengths were applied for intervals of 0.01 to 0.33 second to a definite anatomical spot of the ventricular surface. Such stimuli were given every sixth beat in order to allow full recovery in rhythm and dynamics of cardiac action. The closing and opening of the circuit were arranged so as to advance or recede slightly with respect to the cardiac cycle in successive stimulations. In the majority of experiments, bipolar, ball-tipped, Ag·AgCl electrodes about 8 mm. apart and prepared after the method of Langelaan (3) were used. Such solid electrodes have the advantages 1, that they can be pressed firmly against the ventricular surface without causing mechanical stimulation, with avoidance of a variable "apparent" resistance with movements of the heart, and 2, that they offer less resistance to direct current than fluid electrodes, thereby reducing the voltages necessary for fibrillation and diminishing the tendency for currents to spread.

Unfortunately, with potentials of 10 volts or over, and particularly when durations of current equal 0.10 second or more, slight polarization does occur. Cumulative effects of repeated stimulations were avoided by meticulously changing the direction of current in successive stimulations by an automatic pole changer.

The duration of the rectilinear shocks and their progressively changing relation to the cardiac cycle were determined by a rotating stimulator (fig. 1, *R*), set to operate ever so slightly out of phase with the heart beat. The speed of the rotator was first set by applying very brief weak shocks (ca, 1 M.A./0.02 sec.) to the ventricle, until a whole series (7–15) caused no effect and another similar series of shocks all evoked premature contractions. When records were taken, it was then found that the former fell during successive moments of systole and the latter during progressive moments of diastole. As a rule, a succession of 9 or 10 effective and the same number of ineffective shocks were sufficient for exploratory coverage of the cardiac cycle. When the tempo of the stimulator had thus been set, the duration of the shock was set by means of the rotating cylinder, *R*, with a longitudinal V-shaped gap which broke the contacts of two smooth tungsten surfaces for variable intervals. Such an initial opening rather than

closing current was employed because it is mechanically much easier to open a circuit than it is to close it evenly for a brief and constant interval.

The difficulty of sparking and uneven contact surfaces, which so easily frustrates mechanical production of pure rectilinear shocks, was overcome by passing very small currents with rather high voltage through these contacts. As illustrated in figure 1, this was done as follows: During the stimulation interval desired, the rotating cylinder, *R*, broke the circuit of a bias voltage (300 volts) across an RCA 885 gas triode. As long as the contacts remained closed, the tube was so biased that it did not conduct. With the break of the bias voltage through the rotating stimulator, instan-

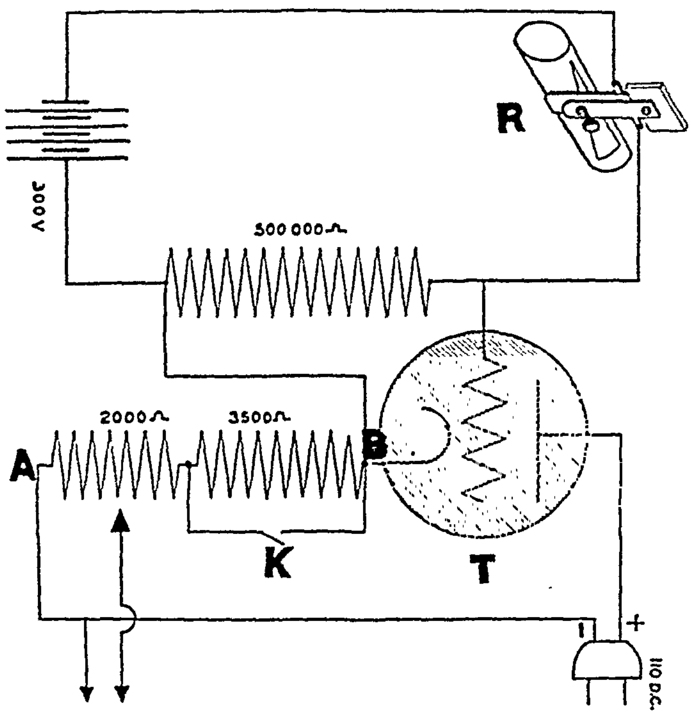


Fig. 1. Schematic diagram of stimulator used. Description in text.

taneous ionization of gas in the triode permitted the tube to conduct a current with a voltage drop of 85 to 90 across the terminals *A*-*B*. As soon as the bias current was completed again by the rotating stimulator, it ceased to conduct immediately, because the high grid potential (300 V.) was sufficient to cause immediate de-ionization of the gas in the tube. In this way, the duration of conduction by the triode synchronized with that of the contact breaker.

The voltage across *A*-*B* was reduced to suitable stimulating values by a voltage divider. By closing a key, *K*, voltage ranges from 2 to 80 could be obtained, and by opening this key small variations between 0.1 to 2 volts were realized. The latter proved useful for tests in relating the rhythms of the stimulator and heart.

A left intraventricular pressure curve and a standard electrocardiogram lead were optically recorded by usual methods. A Wiggers type of manometer was used for the former, and a G.E., type A, amplifying electrocardiograph, the galvanometer of which was removed and arranged to write with the pressure curve on 12 cm. film, was found to have decided advantages over string galvanometers in recording the electrocardiograms.

The moment of stimulation was definitely indicated in such electrocardiograms and since temporal relations were chiefly concerned in our studies, this sufficed in our earlier experiments of this series. In later experiments, a G.E., PM-10 oscillograph was introduced into the circuit. This was so arranged that it recorded a 1 mm. deflection per M.A. current, thus giving quantitative data regarding the stimuli used.

Repeated studies of fibrillation—up to 41 in one dog—were made possible by applying as promptly as possible 1 to 7 brief alternating current shocks in succession through padded electrodes. In 225 trials, in this series alone, we had only one failure. It was gradually discovered that the electrodes did not need to be left in place, as reported in a previous paper (2), a matter of importance in recording simultaneous electrocardiograms during observations. As a rule, normal beats were restored after about 1 minute of fibrillation, but we waited an additional 15 to 20 minutes before another test was made.

Results. In order to present our analysis of miles of optical tracings as succinctly and clearly as possible, we are adopting the expedient of referring first to the chart of figure 2 which summarizes our main deductions. We shall follow this with segments of illustrative records, which authenticate our chart, despite the necessity of reducing their size significantly for publication. It may be added that these reproductions of actual records do not represent consecutive beats in any experiment but merely sections of such records, occasionally rearranged slightly in sequence for the sake of logical presentation. This accounts for the apparent failure in reversal of D.C. shocks in the successive segments selected. For the purpose of this presentation, the results are best arranged from the dominant viewpoint of the duration of application rather than intensity of current, it being understood that only currents which are suprathreshold as regards induction of premature contractions or fibrillation are being considered, unless otherwise specified.

I. Short D.C. shocks (ca 0.01 to 0.04 sec.) behave as unitary excitants to the ventricle, i.e., they cause responses which cannot be differentiated from those of brief induction shocks or condenser discharges. In other words, we have found no evidence that *closing* (C) or *opening* (O) of the current have separate stimulating values. This may be due to the fact that the stronger C effect causes a response and the succeeding O effect invariably comes during the refractory period.

As illustrated by line I of figure 2, such shocks applied during the refractory period of systole, *a*, are ineffective regardless of strength.[3] Applied during the vulnerable period, *b*, i.e., during the last 0.03 to 0.06 sec. of mechanical systole and sometimes during the first 0.02 sec. of protodiastole, they elicit an early diastolic premature contraction when they are weak or induce fibrillation when sufficiently strong. The currents which

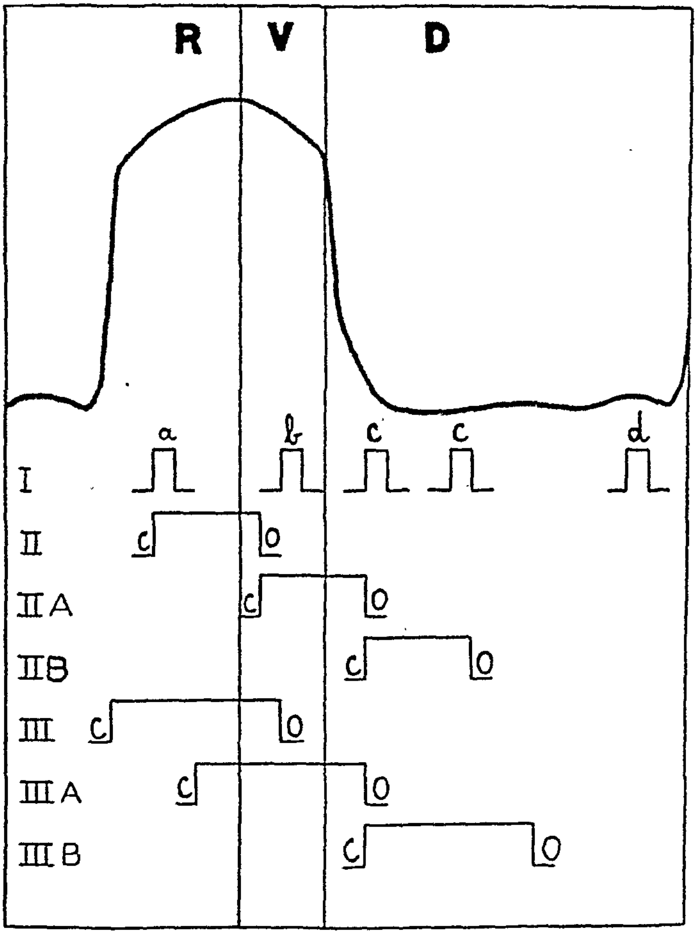


Fig. 2. Diagram indicating the placement of D.C. shocks with reference to a cardiac cycle indicated by a left ventricular pressure curve (upper). *R*—refractory period; *V*, vulnerable period; *D*, diastole. *C*, closing; *O*, opening of D.C. shocks. Further discussion in text.

applied to one locality of the ventricle produce fibrillation, vary slightly in different dogs but remain remarkably constant in repeated trials on the same heart. When such shocks are applied during diastole, *c*, they invariably cause a premature beat, *but never fibrillation.* When such shocks

[3] This statement should be amended to the effect that in 3 experiments out of 90, strong shocks were occasionally effective during this period. In correspondence, King states he has seen a similar undoubted effect.

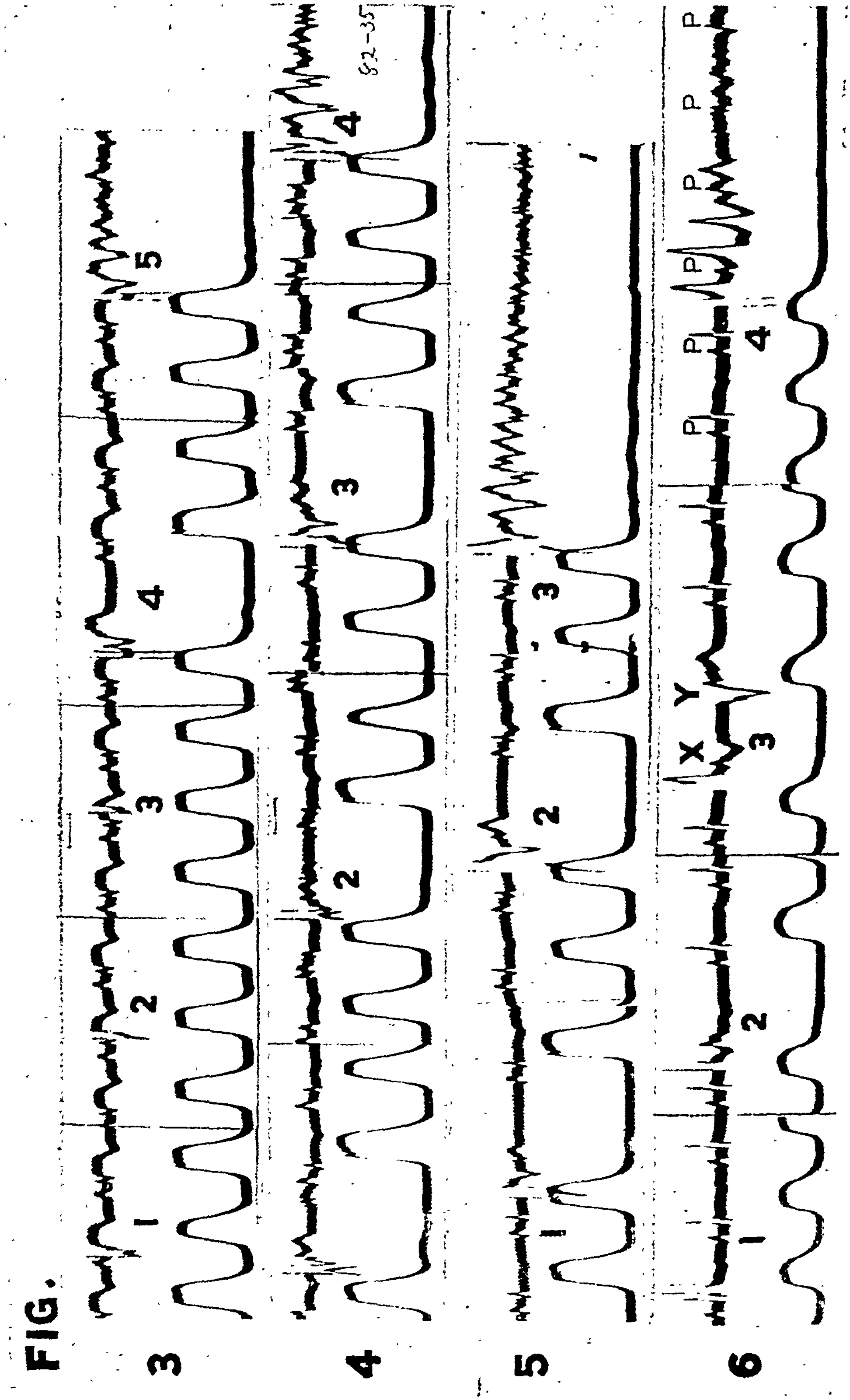
are applied late in diastole, *d*, (i.e., temporally, after the summit of the P wave of normal beats), they are ineffective. This pseudo-refractory phase of late diastole has already been analyzed by one of us (4).

Figure 3 shows segments from an actual record which illustrates some of these reactions induced by shocks 0.04 second in duration. The shocks numbered consecutively show the following reactions: Shock 1 (12 M.A.) applied in diastole causes a large premature beat. Shock 2 (12 M.A.) applied during presystole or pseudo-refractory period of diastole is ineffective, as is shock 3 (12 M.A.) during the refractory period of systole. Shock 4 (12 M.A.) during the vulnerable period gives a premature beat; shock 5 (12 M.A.), a trifle later in the vulnerable period, results in fibrillation. Apparently 12 M.A. represents a critical current for induction of fibrillation. Currents below this were ineffective throughout the experiment.[4]

II. Direct currents of somewhat longer duration (ca. 0.05-0.09 sec.) frequently produce effects comparable to those of shorter shocks. However, there are conditions in which closing (C) and opening (O) effects of the current become operative. As illustrated schematically by line II of figure 2, when *C* occurs during the refractory period and *O* during the vulnerable period, *O* causes a premature systole or fibrillation, depending on the intensity of the current. If, as illustrated, by line II, A, *C* occurs during the vulnerable period, *C* produces similar effects when currents are strong. In the case of weaker currents which only cause a *C* premature beat, *O* has no effect because it falls during the refractory phase of the premature beat. With still weaker currents, *C* causes no effect during the vulnerable period, but if *O* falls during early diastole, premature contraction results. When *C* and *O* of such a current occur exclusively in diastole, as illustrated in line II, B of figure 2, only a premature *C* contraction is induced.

The curves of figure 4 illustrate the effects of 17 M.A. currents, 0.05 second in duration, when they occur progressively earlier in the natural heart cycles. Shocks 1 and 2, in early diastole, cause a feeble premature beat as does shock 3, which is placed in the vulnerable period. Shock 4 falls a

[4] It will be noted that the natural E.C.G. waves exhibit a pronounced monophasic tendency, which developed suddenly after one of the preceding defibrillations. While this did not affect the results, we have chosen this record for the lesson it teaches. During the course of the observations, the notation was made that the E.C.G. suggested a posterior injury. At autopsy, it was discovered that the diaphragmatic wall of the heart had been burned by the unprotected defibrillating electrode. These were employed in our earlier experiments since no harmful effect was discovered. In this case, however, the edge had apparently come into contact with the heart, causing the burn. Subsequently we employed only padded electrodes soaked in saline and suggest this precaution to those who have occasion to use such electrodes.



Figs. 3-6

little later and produces fibrillation. In figure 5, shocks 1 and 2 of 15 M.A. and 0.05 second in duration fall during the vulnerable period and produce a single premature contraction; shock 3, which falls during a similar period and has the same duration but a strength of 17 M.A., causes fibrillation.

Figure 6 shows that strong shocks (e.g., 15 M.A., 0.05 sec. in duration) occasionally elicit still other reactions, not seen with briefer ones. Shock 1 falls during the refractory period and is ineffective. In shock 2, *C* is too early to be effective; and *O* produces a premature systole. In shock 3, *C* occurs during the vulnerable phase and *O* in early diastole. The immediate effect is an artificial premature beat *X* which causes an insignificant pressure elevation. This, however, is followed later by another spontaneous[5] premature beat *Y* which, to judge from the E.C.G., arises from a different focus. Shock 4 falls entirely during the vulnerable phase and produces the anticipated fibrillation.

III. Currents which have a minimal duration of 0.10 to 0.14 second may cause fibrillation when *C* or *O* occurs during the vulnerable period. In addition, they have the inherent possibility that both *C* and *O* of the current may cause excitation during non-refractory periods.

As illustrated in figure 2, line III, when *C* occurs during the refractory period and *O* during the vulnerable period, the latter causes fibrillation when the current is strong, otherwise a premature contraction. However, this may be followed by another spontaneous contraction. If, as illustrated in figure 2, line III, A, *C* occurs early in systole and *O* during early diastole, several results may appear: 1, A spontaneous beat[5] may occur while the current remains closed; 2, *O* may induce a premature beat or 3, both may occur. In the latter case when *O* falls during the vulnerable period of the spontaneous premature beat, fibrillation may result. We can therefore understand why *O* of a prolonged direct current extending into diastole can cause fibrillation. If the relation between the duration of the D.C. shock to the incidence or duration of the premature contraction alters ever so little, fibrillation does not occur. Thus, if *O* falls during its refractory phase, it has no effect; if it falls during relaxation, another premature beat results.

If, as exemplified in figure 2, line III, B, a direct current 0.10–0.14 second in duration is applied during diastole, *C* causes a premature beat and the effects of *O* depend on its incidence during the refractory, vulnerable, or diastolic phase of the *C* premature beat. In other words, such currents may cause one premature beat, fibrillation, or two premature beats. We are thus able to understand why more prolonged shocks during diastole may fibrillate, whereas brief ones cannot accomplish this. It also makes it obvious why the effects of more prolonged shocks cannot be predicted or,

[5] For lack of a better designation, we refer to premature beats as "spontaneous," when they do not definitely follow *C* or *O*.

when they occur, cannot be evaluated without analytical material such as we are presenting. Finally it should be clear that such fibrillating potency disappears again when D.C. shocks are lengthened still more.

In figure 7, currents of 9 M.A. and 0.10 second duration were used throughout. In shocks 1 and 2, *C* occurs early in diastole, causing a premature beat; *O* falls during its refractory period. However a spontaneous premature beat occurs later. In shock 3, *C* occurs during the vulnerable period causing an artificial premature contraction and *O* in early diastole, a second one. Obviously, such reactions show clearly that *C* of such a strength current, when applied during the vulnerable period, is below the fibrillation threshold. But when, as in shock 4, during the vulnerable period *C* causes a premature contraction and *O* presumably falls during the vulnerable period of this premature beat, the two effects together eventuate in fibrillation.

In figure 8, from the same experiment, direct currents of 9.5 M.A. were applied for slightly varying intervals indicated on the curve. Shock 1 is followed by a single premature beat which probably arises spontaneously. Shock 2 is followed by a single contraction, more probably due to *O*. Shock 3 causes a premature *C* and a premature *O* contraction. Shock 4, which covers a large part of systole with *O* occurring in early diastole, leads to fibrillation.

In the experiment of figure 9, shocks of 5 M.A. were applied for 0.14 second so that they advanced slightly with respect to heart cycles. Shock 1 induces only an *O* premature beat. In shock 2, *C* causes a premature beat in early diastole, *O* being ineffective during the refractory phase. In shock 3, *C* causes a large premature beat later in diastole while *O*, falling in the vulnerable phase of this beat, causes another small premature beat. In shock 4, *C* similarly causes a premature beat and *O*, presumably falling in the vulnerable phase of this beat, causes fibrillation. Similar reactions to long diastolic shocks are shown in beats 5 and 6.

A glance at the pressure waves of premature beats following *C* shocks 1, 2, 3 and 4 reëmphasizes that the coincidence of an *O* shock with the vulnerable period of these beats depends not solely upon the time difference between *C* and *O*, but also upon the latency of the premature beat and the time that its summit is reached. Both are conditioned by the time of diastole in which a premature beat is induced. (For details cf. Wiggers (4).) However, the fact must be faced squarely that we cannot relate the vulnerable phase of premature ventricular beats as definitely to ventricular pressure curves as is possible in normal beats. This is illustrated by records of figure 10 in which currents of 9 M.A. and 0.12 second in duration were opened at the summit of a *C* premature beat in shocks 1, 2, 3 and 4. In shocks 1 and 3, *O* was ineffective; in shock 2, *O* caused another premature beat and in shock 4, *O* led to fibrillation. In other words, the heart was

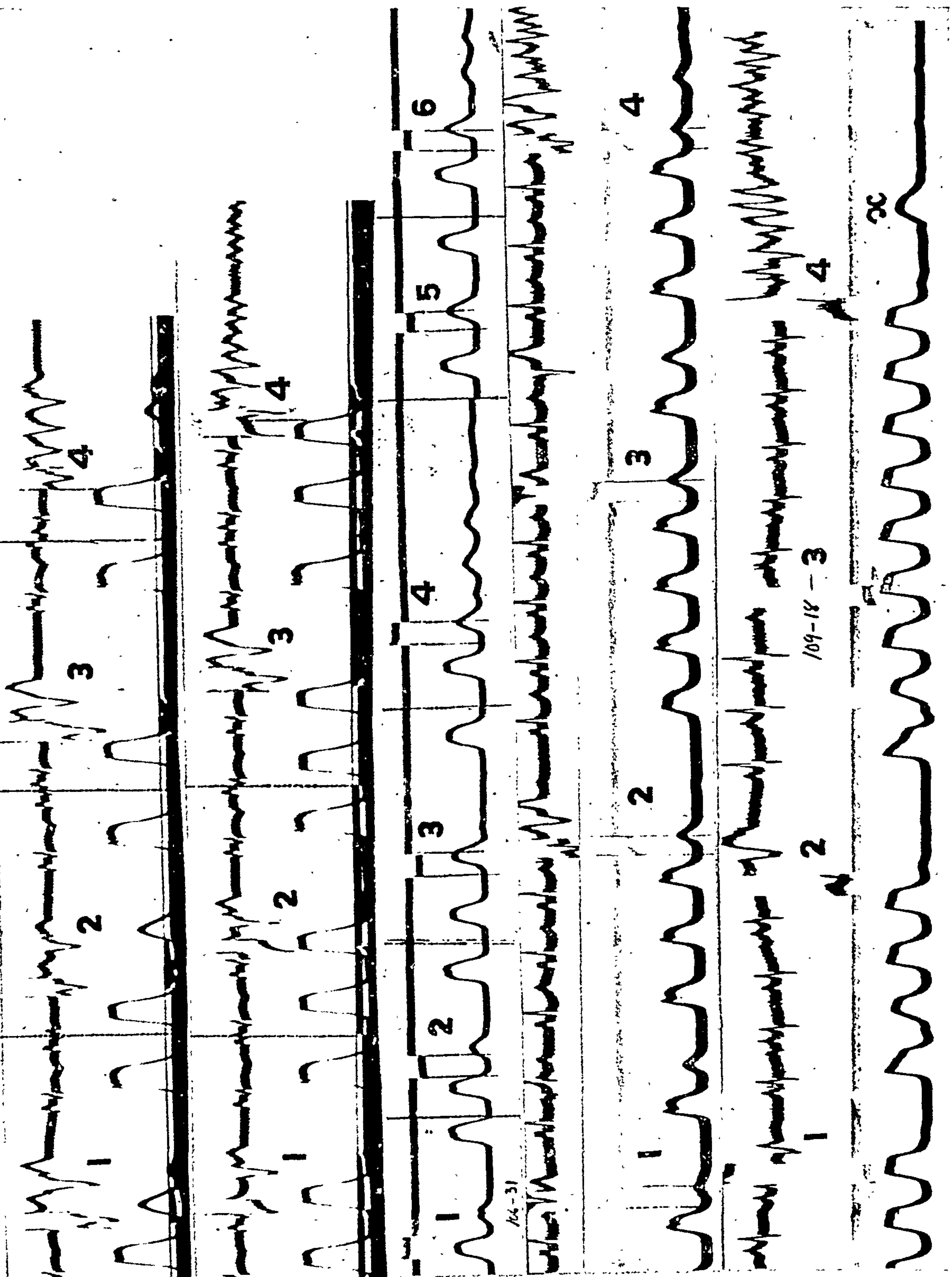


FIG.
7
8
9
10
11

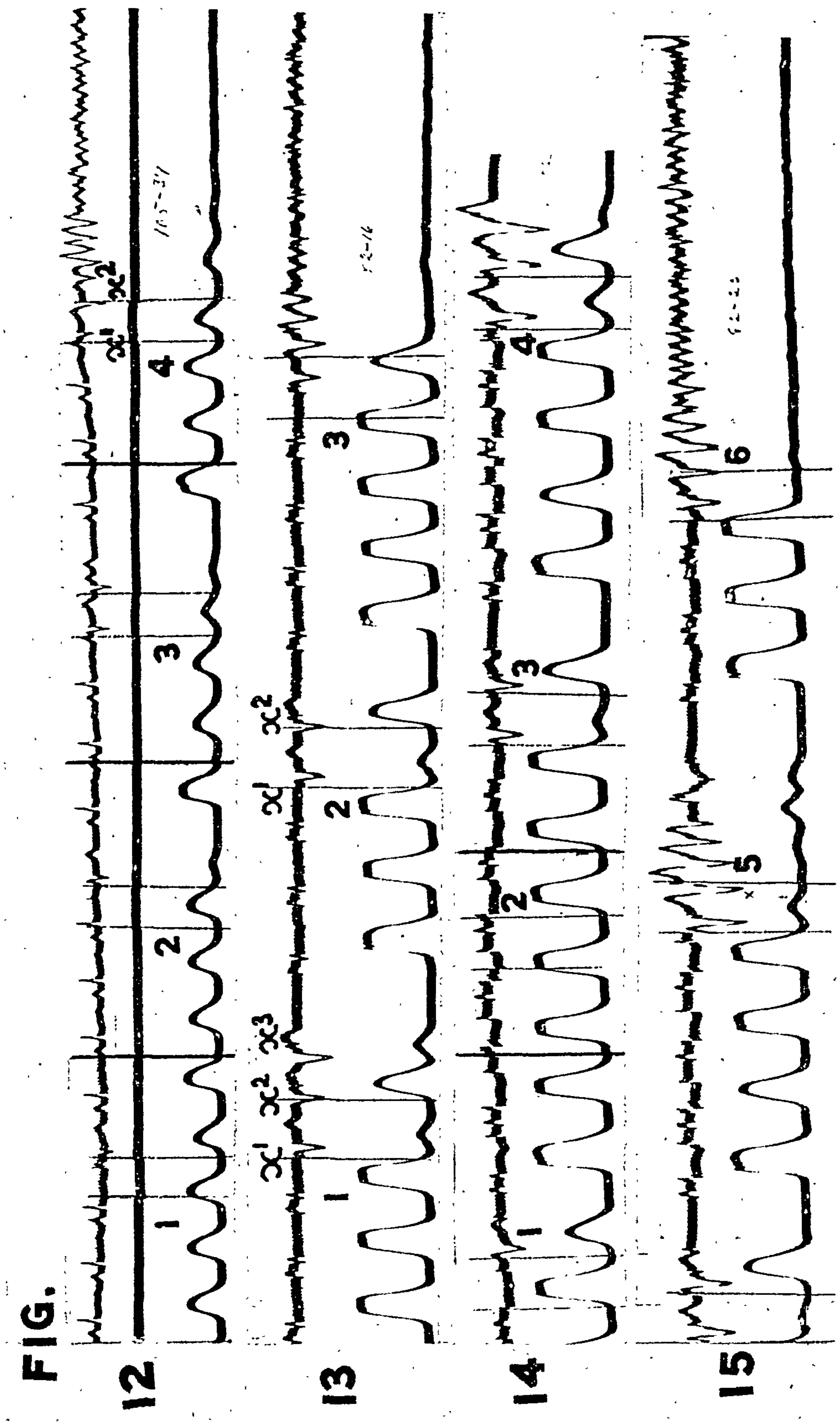
respectively refractory, vulnerable and reactive to diastolic stimuli at the summit of a pressure curve. This is not surprising in view of the fact that with aberrant excitation involved in premature beats, the ventricular pressure curve represents the resultant of many fractionate contractions which neither start nor end in phase. Consequently, the summit bears a very variable relation to the vulnerable phase of the fibers at any point selected for stimulation.

Furthermore, there is a great deal of variability in relation to the amplitudes of E.C.G. deflections and pressure variations in premature beats evoked under different circumstances. This is well illustrated in various records presented, e.g., figure 3, 1 and 4; figure 6, 3, *X* and 3, *Y*; and figure 7, 1 and 2. The pressure variation that occurs during the initial stages of fibrillation shows even more variability in relation to the E.C.G. deflections. As a rule, they show very small pressure variations, as illustrated in figure 3, *5*; figure 4, *4*; figure 5, *3*; figure 6, *4*. Occasionally a number of definite elevations occur giving the erroneous impression of coördinated beats, belied by the E.C.G., as was the case in figure 10, *4*. To this may be added figure 11, *4*, in which fibrillation caused a most unusual pressure variation x, while the E.C.G. was totally irregular.

These difficulties in defining any vulnerable state of premature contractions is discussed at length in order to emphasize the difficulty of presenting crucial proof for our probable interpretation that direct currents applied during diastole can only cause fibrillation when *O* falls during the vulnerable period of a premature beat induced by *C* or arising spontaneously during passage of the current.

IV. Direct currents longer than 0.20 second may exert many of the actions of currents with shorter durations. In addition, they may cause fibrillation at current intensities that are very small. Figure 12 shows a record in which direct currents of only 1 M.A. were applied at different temporal relations to heart cycles for 0.25 second each. Closure of such feeble currents during the vulnerable period is ineffective. Stimuli 1 and 2 show that *O* during diastole causes a premature beat. In beat 3, *C* and *O* during diastole each give a premature response. A similar incidence of *C* and *O* stimuli at 4 leads to fibrillation, however. An examination of the E.C.G. shows clearly that the first premature systole x^1 is doubtless artificial and due to *C*; the second x^2, however, starts before *O* and is therefore probably spontaneous. If it be assumed that this spontaneous premature impulse comes during the vulnerable period of the first, the fibrillation is accounted for in a logical manner.

In figure 13, a direct current of about 1 M.A. was periodically applied for 0.33 second. Stimulus 2 causes at *C* an artificial premature beat x^1 plus a spontaneous beat x^2. Stimulus 1 reacted likewise but, in addition, evoked another spontaneous beat x^3 some time after *O*. These records



Figs. 12-15

illustrate that the tendency of the myocardium to develop spontaneous beats increases as the duration of the stimulus increases. With long applications, e.g., 1 second, it may evoke a continuous series of such beats and, if opening falls at a fortuitous moment, fibrillation results. Fibrillation also results when a spontaneous beat occurs after *O* at the vulnerable period of an *O* premature beat. Stimulus 3 was fortuitously so applied that *C* and *O* fell precisely in vulnerable periods. It is improbable that the *C* shock of 1 M.A. could itself have induced fibrillation; but when *O* comes during a vulnerable phase of the premature beat it elicits fibrillation. This and similar observations make it probable that the fibrillating threshold of aberrant beats is lower than that of normal beats; but the question requires more thorough study.

Figures 14 and 15 show compact illustrations of the different effects which can be anticipated from a 6 volt current, 0.33 second in duration. These are: No effect (stimulus 2); one premature systole (stimulus 1); two premature systoles (stimulus 3); three premature beats (stimulus 4); a run of premature beats (stimulus 5) and fibrillation (stimulus 6). In the last event, closure falls during the vulnerable period, but it is unbelievable that closure of such a weak current alone was responsible for the fibrillation.

A note on chronaxie of the ventricles. It is not our purpose to discuss in detail the validity of chronaxie determinations of the mammalian ventricle. However, our observations clearly indicate the inherent difficulty of determining a rheobase, for a direct current over 0.05 second in duration can cause a multitude of effects, and if applied at appropriate moments, may even fibrillate the ventricles. Attempts to establish a rheobase in the beating heart by using the development of premature beats as a criterion, without regard to the incidence of *C* and *O* of such currents and without evidence as to whether such beats are effects of *C* and *O*, or arise spontaneously, seem to us a valueless procedure. This, together with the fact that the beating heart, unlike nerve or resting skeletal muscle, has no span of iso-excitability during any part of the cycle, seems to invalidate present methods for determining chronaxie as a measure of ventricular excitability.

SUMMARY AND CONCLUSIONS

This research was designed to establish, as far as possible, the conditions under which ventricular fibrillation is produced by direct currents of different durations and to offer a logical explanation for its occurrence. For this purpose D.C. shocks ranging from 1 to 50 M.A. were applied for intervals of 0.01 to 0.33 second to a small area of dogs' left ventricles by nonpolarizable electrodes. Shocks were introduced in alternating directions at every 6th ventricular beat and were so spaced that they fell progressively earlier or later in relation to normal cycles. In this way, the incidence of closing and opening as well as duration of action could be established. Our analysis leads to the following conclusions:

1. The dominant factor which determines the induction of ventricular fibrillation by any electrical stimulus is the fact that any type of stimulus above a certain critical value, introduced during the vulnerable period of late systole, causes simultaneously a premature contraction plus some local or generalized disturbance of conduction which permits irregular reëntry of impulses and leads to the various stages of fibrillation described by one of us (5).

2. In the case of direct currents, the effective excitant may be (a) a brief rectilinear shock, probably not more than 0.04 second in duration or (b) the closing or opening of more prolonged currents during this period of vulnerability.

3. In the case of D.C. stimuli which exceed 0.05 or 0.06 second, several secondary factors may enter which also lead to fibrillation.

The factor which determines fibrillation in any specific instance depends upon the duration of the D.C. stimulus, upon the time that closure occurs with respect to the cycle, upon the character of the premature response evoked, upon the relation of opening to a normal or premature beat, etc. However, we believe that it can be demonstrated in many of these highly variable conditions, and is probable in others which cannot be so definitely analyzed, *that a second effective stimulus, artificial or physiological,* must strike during the vulnerable period of a premature systole in order to cause fibrillation. Such a concept harmonizes the apparent discrepancy that brief shocks of any form only cause fibrillation when they are applied during the vulnerable period, whereas D.C. shocks longer than 0.05 second may do so, when they fall entirely in diastole, partly in systole and partly in diastole, or even when they extend over several beats.

4. The chief secondary mechanisms by which shocks longer than 0.05 or 0.06 second become more potent fibrillation stimuli, are fundamentally due to two well known peculiarities of such shocks: 1. They are no longer unitary stimuli but are capable of exerting separate *C* and *O* effects, and 2, they cause effects (electrotonic?) during persistence of the current, which give rise to unpredictable spontaneous impulses either during the flow of the current or shortly after its cessation.

5. The manner in which such secondary phenomena lead to fibrillation with currents of increasing duration (0.07–0.33 sec.) is graphically depicted in figure 2. In a broad way, these conditions can be resummarized verbally as follows:

(a) When *C*, falling during the vulnerable phase or early diastole, causes a premature contraction of such duration that *O* of effective strength occurs during its vulnerable period.

(b) When *C* occurs during the refractory period of a normal cycle, but the continued passage of the current causes a spontaneous premature beat in the next or any subsequent diastole and *O* occurs during its vulnerable period.

(c) When C occurs during the refractory period of a normal cycle and O falls during early diastole of this or any subsequent beat causing a premature contraction *and* later a spontaneous premature impulse which coincides with the vulnerable period of the first premature beat due to O.

6. In view of (a) *the greater tendency even of weak currents*, 0.24–0.33 second in duration, to evoke spontaneous premature beats not related to C or O; (b) the frequency of fibrillation by such currents in our series and (c) the non-existence in the beating heart of any span of iso-excitability, the determination of chronaxie of the ventricles by use of direct currents introduces difficulties.

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PRODUCTION OF VENTRICULAR FIBRILLATION BY ALTERNATING CURRENTS[1]

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In the preceding paper, we analyzed the effects of localized direct currents of different durations upon the dog's ventricle, with special reference to the fundamental conditions under which they produce fibrillation. This communication deals with a similar analysis of the effects of alternating currents, based on many observations upon 21 dogs.

The anesthesia, operation, method of defibrillation and experimental procedures were the same, except that an electromagnetic key operated by the cylinder contact-breaker, *R*, needed to be substituted for the gas triode, *T*, of figure 1 in the previous paper. While 60/second sine waves probably do not constitute the most effective stimuli, our studies were largely restricted to such a frequency, owing to the practical relation of such currents to production of fibrillation. The currents employed were either a house current, reduced to proper voltage by means of a rheostat, or that produced by a G.R., type 377, oscillator. Left intraventricular pressure, a standard E.C.G. and an oscillographic tracing of the A.C. stimulus constituted our routine recordings. Unfortunately, in the great reduction of records, the sine waves are not always reproduced clearly but the onset and offset are clearly marked. One or the other of these records is occasionally omitted in reproduced curves to conserve space.

Results. Although the conditions under which 60 cycles alternating currents cause fibrillation are very similar to those under which direct currents are effective, it is important that these facts be critically established. In addition, the study of such records brings out many significant observations which must be added to our stock of facts upon which a theory of ventricular fibrillation may ultimately be based.

Stimuli composed of ½ to 4 waves (0.0083 to 0.0666 sec.), like brief D.C. shocks, act as a unitary stimulus, for while each wave is a potential stimulus, only one can be effective, since the waves following the effective one

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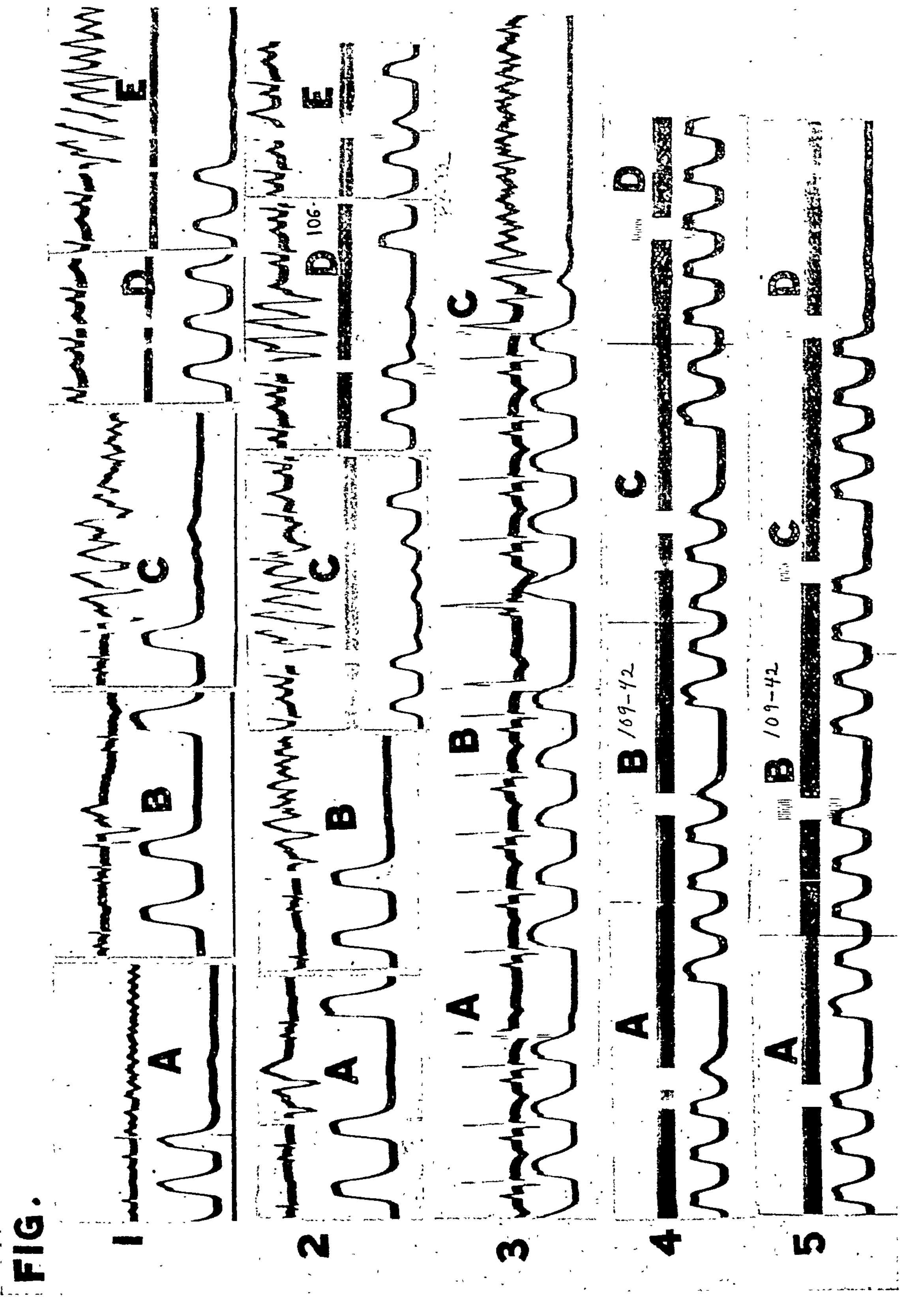
fall during the refractory period of any premature beat evoked. Generally this can be extended to A.C. stimuli of 5 or 6 sine waves; but under exceptional conditions these may evoke a double response, conveniently discussed with somewhat longer shocks (see below).

Even one-half of a 60 cycle sine wave—the shortest attainable with our apparatus—causes all the effects of 3 or 4 such waves. As shown in figure 1, A, if strong enough and applied during the vulnerable phase of late systole, it causes fibrillation. However, such partial waves, like one whole wave, may, at critical voltages, produce either a premature beat, as in 1, B, or fibrillation as in 1, C. The possibility exists that this is due to the phase angle at which the stimulus begins, a question that we were not in a position to investigate thoroughly. A glance at the original records shows a phase start at approximately 0° in 1, B, and about 220° in 1, C.

Two or three sine waves of sufficient voltage cause fibrillation when they fall strictly within the vulnerable period or start in this period, as shown in figure 1, E. During the refractory phase, as shown in figure 1, D, they evoke no response. In these, as in experiments with brief condenser and D.C. shocks, it is extremely difficult to ascertain the exact span of the vulnerable period. For example, we have found occasionally that a stimulus applied during early diastolic relaxation causes fibrillation and we have periodically been inclined to include this in the vulnerable phase. The records of figure 2, A and B, are interesting in showing that this is not universally true, or at least that the last moments of systole seem to be more highly vulnerable. In both cases, 3 sine waves of the same strength fall toward the ends of vulnerable periods, but sufficiently later in 2, A to overlap slightly into the phase of isometric relaxation. Fibrillation occurs only in 2, B. Such observations become significant only when repeated. Therefore, figure 3 is also presented, for it shows beautifully the clear demarcation of the vulnerable phase at the moment when isometric relaxation occurs. In figure 3, A, B, C, three sine waves fall ever so slightly earlier in the cycle; but only the last stimulus which actually starts during late systole induces fibrillation.

As the number of waves increases, the chance of diastolic overlap becomes greater. In figure 2, C and D, five waves falling partly in the vulnerable phase and partly in early diastole give rise to a series of reentrant waves of excitation shown in the E.C.G.; whereas the same series falling solely in diastole evoke a single premature beat only (fig. 2, E). Such a series of reëntrant waves represents a potential fibrillation. They are very like those which initiate fibrillation, e.g., as illustrated again in figures 1, C, 1, E, 2, B and 2, C. Why fibrillation does not eventuate must be studied further, but it stresses the fact that any satisfactory theory of fibrillation must explain *a*, the initiation of a premature contraction and a series of reëntrant waves, and *b*, the factors which favor their transformation into true fibrillation.

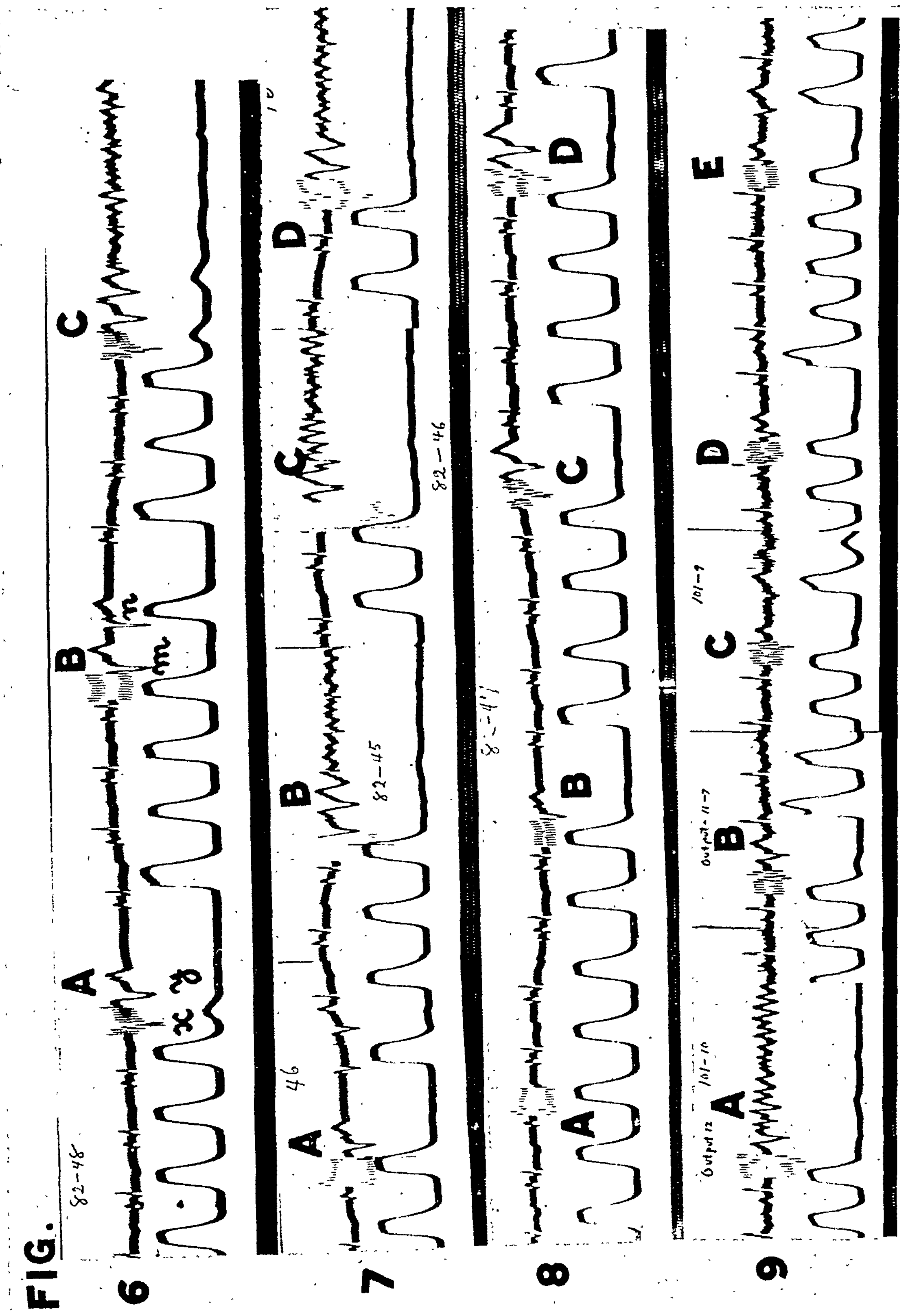


Figs. 1-5

A.C. stimuli containing 7 to 9 60/second sine waves (0.116 to 0.15 sec.) produce a variety of effects depending upon their strength and placement in the cardiac cycle. When they are very weak, they are without effect or produce an occasional premature contraction. In figures 4 and 5, a series of 9 larger waves falling frankly in diastole (fig. 4, A, B, C) evoke two premature contractions, the second of which is barely perceptible in original ventricular pressure curves. This causes an extension of the compensatory pause by the time-equivalent of one normal beat, i.e., interpolation of two premature beats during a normal compensatory pause does not occur. When the waves fall entirely during the refractory period (fig. 4, D), they are ineffective; but as soon as they encroach upon (fig. 5, A), or start in (fig. 5, B and C) the vulnerable phase, they evoke a single premature contraction followed by a compensatory pause. It is important to note that currents of such strength did not cause fibrillation through action during the vulnerable period of late systole. However, if they start ever so early in diastole, as in 5, D, fibrillation results. We may emphasize that with an appropriate number of waves, weaker currents which cannot induce a *systolic type* of fibrillation can cause a *diastolic type*. Of course, if the currents are stronger and fall anywhere in the vulnerable period, they do cause *systolic fibrillation*.

The reason that A.C. stimuli are effective in causing "diastolic fibrillation" is probably found in the fact analyzed in connection with D.C. shocks, viz., that the first of these sine waves is not strong enough to cause such a premature beat when applied during the vulnerable phase, but falling during the isometric relaxation is adequate to inaugurate a premature beat. The train of waves is then long enough so that an effective portion falls during the vulnerable phase of this premature beat. In short, the induction of diastolic fibrillation is determined, as in the case of direct currents, by *a*, the start of the wave series with respect to the cycle; *b*, the character of the premature response evoked, and *c*, the relation of an effective portion of such a series to the vulnerable period of a premature beat. In the case of a D.C. shock, this would need to be the opening of the current; in the case of A.C. currents, any phasic change might suffice, thereby increasing the chance of inducing fibrillation by such currents.

How precisely the series of weaker shocks must be placed with reference to a cardiac cycle in order to cause diastolic fibrillation is illustrated in figure 6 in which 11 waves were applied. In A, it merely evokes two premature beats, *x*, *y*, and in C is followed by fibrillation. The superimposed stimuli and E.C.G. of figure 6 show clearly that the effective moment of a series of sine waves which elicits the premature beat—regardless of whether it is followed by reëntry beats and fibrillation—may be the onset of such a series as in figure 6, A and C. However, if a reasonable latency be allowed in figure 6, B, it is apparent that one of the intermediate waves must have



Figs. 6–9

caused the premature systole, *m*. The second one, *n*, on the other hand, appears to be spontaneous in origin. An examination of many curves permits the inference that moderate alternating currents exceeding 0.16 sec. in duration can cause changes leading to such after-effects, much as do direct currents.

A clear instance in which strength of the current is of moment in the production of systolic fibrillation by a series of 10 or more waves is presented by curves of figures 7 and 8 from the same animal. In each case, 11 sine waves were applied. Those of figure 7 had a strength represented by an output of "12" on the G.R. oscillator and those of figure 8, an output of "10." In the former record, systolic fibrillation occurs when the series of waves extend through the vulnerable period (fig. 7, B), when they start in the vulnerable period (fig. 7, C), but not when they end there (fig. 7, A). With weaker currents, only premature systoles occur as in figure 8, B, C and D; while it is ineffective in 8, A. In addition, figure 7, A gives evidence, by a spontaneous premature beat, of some persistent after-effect. The curves of figure 9 show another example in which a series of waves with an output of "12" on the G.R. oscillator causes systolic fibrillation when they cover the vulnerable period (fig. 9, A); whereas a similar series of waves with an output of "11" fails to do so during any phase of systole (fig. 9, B, C, D and E). Consequently, were it not for the fact that a series of weaker sine currents may induce diastolic fibrillation when effective portions strike at appropriate moments of normal and premature beats, the strength of current composed of 10 or 15 waves (ca, 0.25 sec.) might be used to evaluate the sensitivity of the ventricle to fibrillation.

The effects of more prolonged but very weak alternating currents bring to light phenomena which are obscured by the prompt induction of fibrillation in the case of stronger currents. Weak A.C. stimuli (0.5 M.A. to 1 M.A.), when their duration exceeds 0.2 second, evoke a series of aberrant heats shown in figures 10 to 13. While the development of such a ventricular tachycardia by currents of several seconds' duration is well known to experimenters, the reactions have never been fully analyzed, particularly with reference to their transition into fibrillation.

A detailed study of original records, a few samples of which are shown in figures 10, 11, 12 and 13, reveal the following additional details: The tachycardia starts almost immediately after application of weak A.C. The frequency of beats calculated from E.C.G. deflections ranges from 260 to 375 per minute. It is slower than that of the tachysystolic waves (600 per min.) which are introductory to true fibrillation (cf. fig. 13, *X* and *Y*). The frequency is remarkably constant in the same heart, stimulated at the same points. Each electrical deflection is accompanied by a ventricular pressure wave when the frequency is between 250 and 280 (fig. 10); but when the E.C.G. deflections exceed a critical rate, somewhere between 275 to 300 per

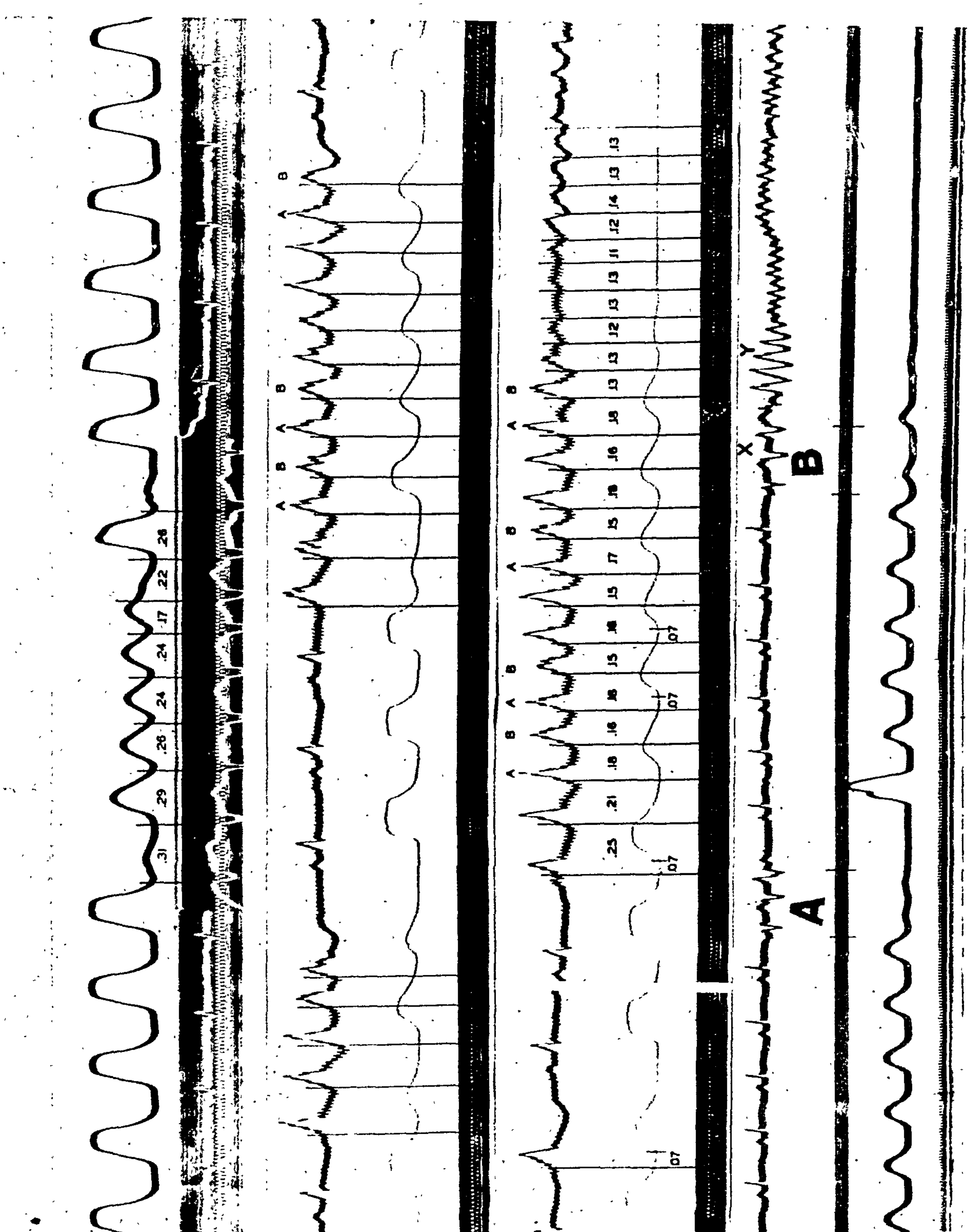


FIG. 10

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minute, the ventricular pressure waves show an alternate lapse (figs. 11, 12). Such dissociation of mechanical and electrical systoles has apparently not been previously described. The spacing of the electrical deflections is rarely even and the corresponding ventricular beats may therefore be variable in amplitude (fig. 10). Different curves have different characteristics. As a rule, the period of the first deflection is definitely longer than that of following deflections. Often a progressive decrease in duration occurs for a few deflections, after which they become more evenly spaced (fig. 11). However, irregularities creep in. Sometimes the electrical deflections are alternately a trifle longer and shorter, and occasionally as shown in beats labeled A and B in figures 11 and 12, the electrical deflections seem to alternate slightly in amplitude. In such cases, the larger deflection is preceded by a shorter cycle and is the one which accompanies a ventricular pressure wave. The ventricular tachycardia induced by very weak A.C. may revert to a normal rhythm shortly after cessation of the stimulation, as in figures 10, 11, 13, A; or it may be converted to fibrillation *either* while current continues to be applied (fig. 12), or shortly after its removal (fig. 13, B). The transition is fairly distinct in either case. It consists in a sudden reduction in the periods of the aberrant E.C.G. waves, e.g., in figure 12 from 0.17 to 0.13 second and in figure 13, B from 0.18 to 0.10 second, i.e., to rates of 460/min. and 600/min., respectively. These beats correspond to the initial stage of fibrillation designated as tachysystolic by one of us. They lead irrevocably into the subsequent transitional stages of true fibrillation.

The question arises, is the tachycardia which occurs during application of weak currents due to release of repetitive stimuli from a focus—presumably in the vicinity of the electrodes—, or to reëntry of impulses? Somewhat in favor of the latter is the fact that an alternate default of left ventricular pressure waves is difficult to explain by repetitive focal stimuli released near electrodes *placed on the left ventricle.* The progressive shortening in periods of E.C.G. deflections is consistently explained by formation of shorter paths of reëntry or progressive increase in conduction rate. However, it can also be explained on the theory of focal stimulation if we assume that passage of A.C. causes a decrease in refractory periods. A certain variability in refractory periods would account for random variability in spacing. Consequently, while consideration of our results favors the concept of reëntry, conclusive evidence cannot be claimed for such a theory.

Why does ventricular tachycardia eventuate in fibrillation in some instances and not in others? We have conclusive evidence that this is not a matter of duration of stimulation, provided the period exceeds only 0.2 sec. (cf. fig. 13, A and B). The mechanisms concerned remain obscure; any ideas are purely speculative. Two speculations may be briefly considered as to probability:

1. In accordance with our general concepts, fibrillation occurs when an effective portion of a stimulus falls during a vulnerable period. The vulnerability may be so enhanced by passage of a weak current that a portion of the series becomes of fibrillating potency. This could not explain fibrillation which follows removal of the current. Again, a natural or ectopic impulse arising at some portion of a reëntrant wave during the local vulnerable period might be strong enough to set off the fibrillation. Careful study fails to reveal convincing evidence of such a possibility.

2. The fibrillation may be unrelated to the focal point of stimulation. Assuming that a series of reëntrant waves occurs over slightly different pathways, the possibility exists that shorter circuits are suddenly formed due to localized variation in refractory periods which lead to the initial tachysystolic phase of fibrillation. Between these and still other speculations, no decision is possible on the basis of available experimental evidence.

SUMMARY

The effects of 60 cycle alternating currents applied locally to the dog's left ventricle through nonpolarizable electrodes are analyzed:

A.C. stimuli composed of ½ to 4 waves (0.008–0.0666 sec.), like brief D.C. shocks, act as a unitary stimulus. When they start or fall entirely within the vulnerable period, they always give a response if strong enough; that response is one or two premature ventricular systoles followed or not by ventricular fibrillation. The influence of the moment of onset or phase angle in relation to the vulnerable period has not been established. A similar stimulus during the refractory period is ineffective. When given during diastole, it causes one premature beat and never produces fibrillation.

Stimuli composed of more sine waves (generally 7 to 9) produce no effect or an occasional premature beat when they are very weak. A similar number, of moderate strength, falling entirely in the refractory phase are ineffective; but when they encroach upon, cover, or start in the vulnerable period, they evoke a single premature contraction, but never cause fibrillation. If they start ever so early in diastole, however, fibrillation occurs. Strong currents also cause fibrillation when they enter during the vulnerable period.

The occurrence of fibrillation by early diastolic shocks, incapable of acting during the vulnerable period, can be explained by the fact that an effective portion of the series now falls during the vulnerable period of a premature beat. In other words, an apparent diastolic fibrillation is actually a fibrillation started during the systolic vulnerable period of a premature beat.

A.C. stimuli, with durations varying from 0.2 to 1 second or more, promptly cause fibrillation when currents are of moderate or great strength.

However, even very weak currents (0.5 to 1.0 M.A.) cause ventricular tachycardia which may revert to a normal rhythm after removal of the stimulus or may eventuate in fibrillation either while the A.C. is operating or shortly after its withdrawal.

An analysis of the ventricular tachycardia reveals that the rhythm is not quite regular and that a tendency to progressive increase in rate occurs. In some instances, electrical alternation exists and a peculiar dissociation of electrical and left ventricular pressure occurs in alternate beats, despite the fact that electrodes are applied to the left ventricle. Therefore, the probability is weighed that the tachycardia is caused by reëntry rather than by periodic focal stimuli. However, conclusive evidence for such a theory is not adduced.

The reason why weak alternating currents produce fibrillation in some tests and not in others remains obscure. However, definite evidence is presented that the duration of such currents is not a factor provided this exceeds 0.2 sec. The mechanisms through which fibrillation eventuates during or shortly after use of such weak A.C. currents are difficult to analyze on the basis of evidence so far available.

THYROID THERAPY AND THE SKULL BONE PATTERN OF THYROPARATHYROIDECTOMIZED RATS

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The functions of thyroid and parathyroid tissue have been extensively studied by many investigators in relation to metabolism, general growth, development of systems and the chemical equilibria in tissues. The problems have been approached by extirpation of the glands separately and together, and by feeding or injecting glandular preparations.

Simpson (1) called attention to the importance of age at the time of thyroidectomy or parathyroidectomy in determining the severity of the deficiency symptoms. He described typical cretins resulting from thyroidectomy at a very young age as being small and stunted animals with broad faces and rickety limbs. Later Basinger (2) described the short extremities of thyroidectomized rabbits as "pseudorickets". That young animals are more susceptible to parathyroidectomy than older ones has been frequently confirmed (3, 5).

The rôle of diet (3, 5) following extirpation of the parathyroid glands has been extensively studied. Patras *et al.* (4) observed that the nutritional condition of the animal in relation to the mineral content of the diet prior to thyroparathyroidectomy, as well as immediately following the operation, contributed to the character of the convalescence.

The importance of environmental temperature (6) and intercurrent disease such as rickets (7), infections, constipation (8) and gastro-intestinal irritation (9) in precipitating tetany from a latent state has been suggested.

Voluminous evidence has been submitted to prove the importance of the parathyroid glands in the development of the skeletal system. Hammett (10) found the bones of parathyroidectomized animals to be low in ash, calcium and phosphorus; and deficiency findings in the teeth have been observed by many investigators (3, 5). Marked decalcification (osteoporosis) has often been observed in clinical hyperparathyroidism and frequently has been produced experimentally by the injection of toxic doses of parathyroid extract (5).

The skeleton is composed of constituents which by the processes of

metabolism are made mobile. Inorganic elements laid down at one time are replaced by incoming elements at a later time. Thus the continuous exchange of inorganic constituents between organized bone and body fluids makes the normal skeletal system an excellent storehouse for emergencies.

The extent to which the detailed patterns of normal bone structure may be altered by the hormones of the thyroid and parathyroid glands is a subject of further investigation together with the relation of this normal pattern to calcium metabolism. Patras, Templeton and Hummon (11) have described a marked disturbance in the mosaic pattern of the skull bones of albino rats following thyroparathyroidectomy. Since the growth of thyroparathyroidectomized animals can be practically restored to normal by the ingestion of desiccated thyroid it seems reasonable to assume that such post-operative treatment might restore the normal bone patterns.

The quantity of thyroid to be used was selected for this work as a result of preliminary experiments in which the growth of thyroparathyroidectomized animals receiving 0.02, 0.05 and 0.1 per cent thyroid respectively in the diets was compared with that of the normal growth of unoperated animals. From this preliminary experiment it was revealed that the group of animals receiving 0.1 per cent thyroid after thyroparathyroidectomy had a high mortality between 100 and 150 days after the start of the experiment. During this period there was a loss of weight in the surviving animals indicating a toxic state. The groups receiving 0.02 and 0.05 per cent thyroid gave growth curves practically identical, and comparable to the growth curve of the normal unoperated animals. Since 0.05 per cent thyroid did not seem more beneficial to growth than 0.02 per cent it seemed advisable to select the smaller concentration for a study of the influence of thyroid therapy on the skull pattern.

For our study on the influence of desiccated thyroid on the mosaic pattern of the skull bones we used 108 albino rats. All animals were taken from the Loyola University stock colony which is maintained on Fox Chow *ad libitum*, with bread and meat twice per week. The rats were weaned at the age of 21 days and thyroparathyroidectomized between the ages of 25 and 31 days. Immediately following thyroparathyroidectomy they were divided into 4 groups. Groups 1 and 2 (23 males and 25 females respectively) received a Fox Chow diet throughout the course of the experiment. Groups 3 and 4 (31 males and 29 females respectively) were given a diet consisting of Fox Chow containing 0.02 per cent desiccated thyroid. All animals were fed their respective diets for 200 days during which time they were weighed at weekly intervals. At the end of the experiment the femurs and skull bones were saved for measurements and x-ray study.

The beneficial effect of thyroid on the growth of the females (fig. 1) and

males became apparent between the 2nd and 3rd weeks of the experiment. This became more pronounced reaching a maximum 70 days after the beginning of the experiment at which time the animals approximated their mature growth. Beyond this time the same effect was maintained throughout the course of the experiment.

The second index was obtained by measuring the femurs after the animals had been sacrificed and the bones cleaned of all attached tissue (fig. 2). The average femur length was found to be 27.9 and 26.2 mm. respectively for the male and female thyroparathyroidectomized rats which did

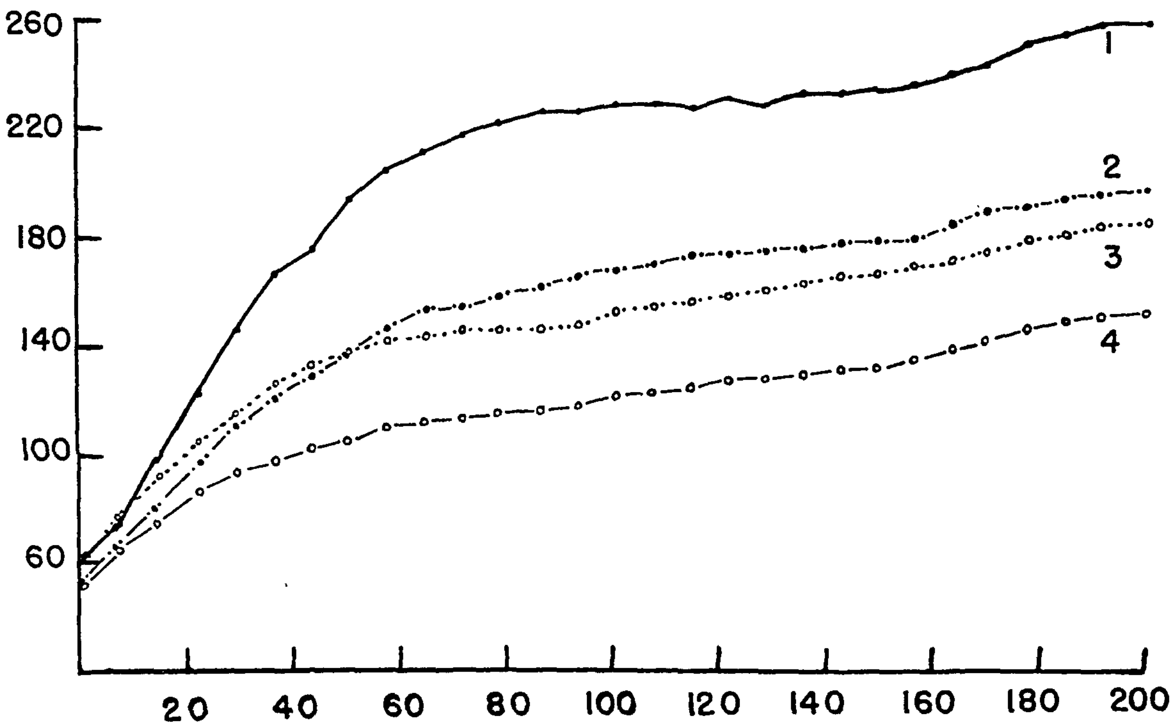

Fig. 1. Weight curve of thyroparathyroidectomized rats. Curve *1*, male rats receiving desiccated thyroid. Curve *2*, female rats receiving desiccated thyroid. Curve *3*, male rats receiving only a Fox Chow diet. Curve *4*, female rats receiving only a Fox Chow diet.

not receive thyroid in the diet. The average femur length was found to be 32.5 and 29.9 mm. respectively for the male and female animals which received a diet containing desiccated thyroid.

The roentgenogram of a normal rat's skull presents a mosaic pattern (fig. 2) which appears to be a calcified network, the interstices of which are much less dense. This normal pattern is obscured after thyroparathyroidectomy by what appears to be a decrease in the density of the network and a slight increase in the density of the interstitial areas. Careful examination of the roentgenograms of the skulls from the experimental animals revealed an increased density (fig. 2) in those groups (groups 3

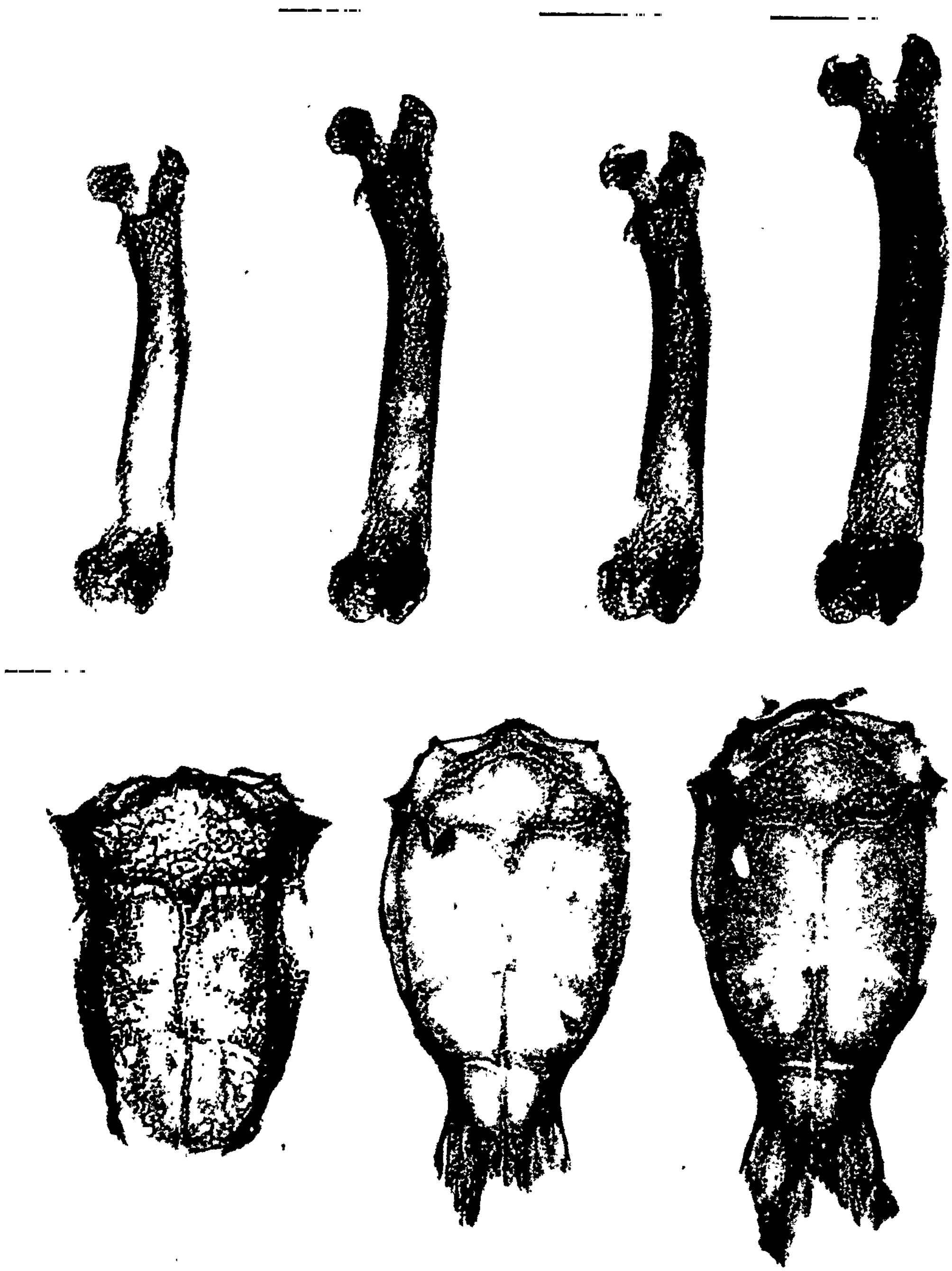

Fig. 2. Femur and skull bones. No. 1, femur of female rat on Fox Chow diet. No. 2, femur of female rat receiving thyroid in diet. No. 3, femur of male rat receiving Fox Chow. No. 4, femur of male rat receiving thyroid in diet. No. 5, skull bone from normal rat. No. 6, skull bone from thyroparathyroidectomized rat. No. 7, skull from thyroparathyroidectomized rat receiving desiccated thyroid in diet.

and 4) which received post-operative thyroid therapy as compared to those (groups 1 and 2) which did not receive thyroid. This increased

density, however, did not seem to alter the obscured details of the mosaic configuration which follows thyroparathyroidectomy.

The mosaic pattern in the thin skull bone is probably concerned with strength. In removing the bones from the animals after death, however, the skulls of those which did not receive thyroid were found to be much more fragile than those belonging to the thyroid-treated animals in spite of the fact that the pattern was not benefitted by the thyroid therapy. This seems to indicate that thyroid feeding is capable of strengthening bone by a mechanism other than the normal, namely, calcification without regard for the normal pattern. Even though the skulls of the thyroid-fed animals were harder and more dense than those of their littermates which did not receive thyroid therapy they were much more brittle and for that reason more fragile than skulls from unoperated animals.

SUMMARY

1. Preliminary experiments on thyroparathyroidectomized rats revealed that Fox Chow containing 0.1 per cent desiccated thyroid caused toxic manifestations after 100 to 150 days.

2. The ingestion of a diet of Fox Chow containing 0.02 and one containing 0.05 per cent desiccated thyroid by thyroparathyroidectomized rats was found not to cause toxic symptoms for at least 200 days.

3. A stimulating effect of 0.02 per cent thyroid in the Fox Chow diet on the growth of thyroparathyroidectomized rats, as determined by the weight curve, was observed.

4. The femur length of thyroparathyroidectomized rats was found to be increased appreciably by the ingestion of a diet containing 0.02 per cent desiccated thyroid.

5. The normal mosaic pattern of skull bones was found to be blurred following thyroparathyroidectomy.

6. The ingestion of Fox Chow diet containing 0.02 per cent desiccated thyroid was not conducive to the maintenance of a normal mosaic pattern in the skulls of thyroparathyroidectomized rats.

7. The ingestion of thyroid by thyroparathyroidectomized rats caused the development of a denser, less fragile skull in which the mosaic pattern was still blurred more than normally.

8. The skulls of thyroparathyroidectomized rats receiving thyroid therapy were more brittle and fragile than those of normal unoperated animals.

We are grateful to R. D. Templeton, R. L. Ferguson and I. F. Hummon for their coöperation in this work.

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STUDIES IN IODINE METABOLISM OF THE THYROID GLAND IN SITU BY THE USE OF RADIO-IODINE IN NORMAL SUBJECTS AND IN PATIENTS WITH VARIOUS TYPES OF GOITER[1]

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A method of studying iodine metabolism by the use of radioactive iodine in a group of patients suffering from several types of thyroid disease was described in a recent report (1). The uptake of administered iodine by the thyroid glands of these patients was determined by the measurement of the radio-iodine content of the extirpated thyroid tissue. This procedure had the disadvantages that only one measurement of the iodine uptake of the thyroid could be made on each patient and that comparable studies with normal controls could not be done. However, in a single experiment the uptake of iodine by the thyroid in situ was determined by the measurement of the gamma rays from the radio-iodine which had accumulated in the glands. By the use of this method the uptake of iodine in the thyroid glands of normal subjects and of patients can be compared and the metabolism of the accumulated iodine in these thyroids can be followed for several weeks after its administration. The present report includes a description of the results of the application of this technic to the study of iodine metabolism of the thyroid gland.

Method of study. The radio-iodine was prepared by the Berkeley cyclotron and converted to sodium iodide by the procedure described in a previous article (1). A solution of sodium iodide, containing 14 mgm. of iodine and an exactly known amount of radio-iodine, was administered to each subject in 100 cc. of water one hour before breakfast. The radioactivity of the radio-iodine administered in each experiment was equivalent to from 12 to 50 μgm. of radium element in equilibrium with its decay products when the gamma rays from these two substances were used as a basis of comparison.

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A scale-of-four Geiger-Müller counter was used for the measurement of the gamma rays emitted from the radio-iodine atoms taken up by the thyroid glands of patients and of normal subjects. In each case the isthmus of the gland was palpated and a small piece of adhesive tape placed over it. *The counter tube employed was 10 cm. in length and 1.5 cm.* in diameter and was encased for mechanical protection in a copper tube whose walls were 1 mm. thick. A mark was made at the center of the copper case so that for each measurement its position would be the same. The radioactivity of the thyroid was determined by placing the counter tube firmly against the gland. The mark denoting the center of the counter tube was placed directly against the piece of tape indicating the center of the thyroid isthmus.

The subject was given the radio-iodine solution to drink and 50 cc. of water were administered to wash all traces of the radio-iodine from the mouth and esophagus, and within five minutes the first determination of the radioactivity of the thyroid gland was made. During the following hour and a half, readings were taken every three to eight minutes. The counter tube was reset against the mark over the isthmus of the gland for each measurement. At the conclusion of the first hour and a half the subject was allowed to leave for breakfast. The determinations were resumed within the next hour and were repeated every hour. The last series of readings for the first day was taken eight hours after the beginning of the experiment. Each series of determinations comprised six to twelve separate readings. These measurements were repeated daily for at least four successive days and, whenever possible, they were continued over a period of several weeks. After the first day only one series of from six to ten readings was taken each day the experiment was conducted.

As has been described previously (2), the measurements made with the counter were corrected for background, decay of the radio-iodine, and nonlinearity of the counter. The sensitivity of the counter was checked with a radium standard several times during each day the instrument was operated. For the six months' period during which these experiments were conducted, it varied less than 3 per cent. Background determinations also were taken at frequent intervals and were observed to vary but slightly. The value of 8.8 days for the half-life was used in correcting for the decay of the radio-iodine.[2] During the first day of each experiment the radiation from the rest of the body[3] made it difficult to determine accurately the radioactivity of the thyroid. However, since the radio-iodine was rapidly excreted (1), this problem became relatively unimportant after the first day.

[2] This value gives the half-life as measured by gamma ray radioactivity of the mixture of radioactive iodine isotopes used in these experiments.

[3] A description of the method of correction for radiation from the body is given in the appendix.

The radioactivity of the thyroid gland in situ was compared with an aliquot fraction of the radio-iodine given to the subject and the center of mass of the gland was estimated by palpation. From these data it was possible to calculate roughly the amount of radio-iodine taken up by the thyroid. Three of the patients underwent thyroidectomy and the radio-iodine content of the removed thyroids was determined by direct measurement using the method described in a previous report (1). A comparison of the values obtained by the measurement of the radioactivity of these glands in situ and after their removal revealed that in two instances the results agreed within 10 per cent. However, in the third case the estimated radioactivity of the thyroid in situ was 50 per cent lower than the direct measurement of the radio-iodine content of the extirpated thyroid. A possible explanation of this discrepancy was that at operation the left lobe of the gland was found to be larger than had been expected and to extend behind the trachea. No corrections were made for the absorption of gamma rays in the thyroid gland and overlying skin and muscle. The gamma rays of the eight-day radio-iodine were observed by Livingood and Seaborg to possess an energy of 0.4 MEV (3) and are therefore sufficiently penetrating to enter the counter tube without significant diminution after passing through the thyroid and the overlying tissues.

Results. The patients studied by this technic included five adults with thyrotoxicosis, two adults with non-toxic goiter and normal metabolic rate, four children with hypothyroidism and without goiter, and one child with a goiter and hypothyroidism. Five normal subjects served as controls. None of the normal subjects and adult patients had received any iodine for at least six months prior to these experiments. The five children with hypothyroidism had received small quantities of iodine in the form of dried thyroid substance until six weeks before the administration of the radio-iodine. The clinical diagnosis was establised in each case by thorough medical examination and complete laboratory studies. The latter included one or more determinations of the basal metabolic rate in all instances and galactose tolerance tests in the thyrotoxic patients. The diagnosis of hypothyroidism in the five children was based upon findings of low basal metabolic rate, elevated blood cholesterol, retarded bone age, and delayed mental development. The five normal subjects had complete medical examinations within a year of these studies and determinations of the basal metabolism at the time of the experiments.

The curves shown in figures 1 and 2 represent the content of radio-iodine in the thyroid glands plotted against time. The abscissae indicate the time in days following the administration of the radio-iodine and the ordinates represent the percentage of uptake of radio-iodine. Because of the limitations of space, only the first five days of the experiments are shown in the figures.

The relative heights of the individual curves of the five normal subjects (fig. 1) show the apparent variations in amounts of radio-iodine taken up by their thyroid glands. These differences are reasonably accurate because the thyroids were roughly similar in size and shape as far as could be determined by careful palpation. The curves closely resembled each other in form although there was considerable variation in their maximum values. In each of the five experiments, the greatest uptake of radio-iodine by the thyroid apparently was not reached until

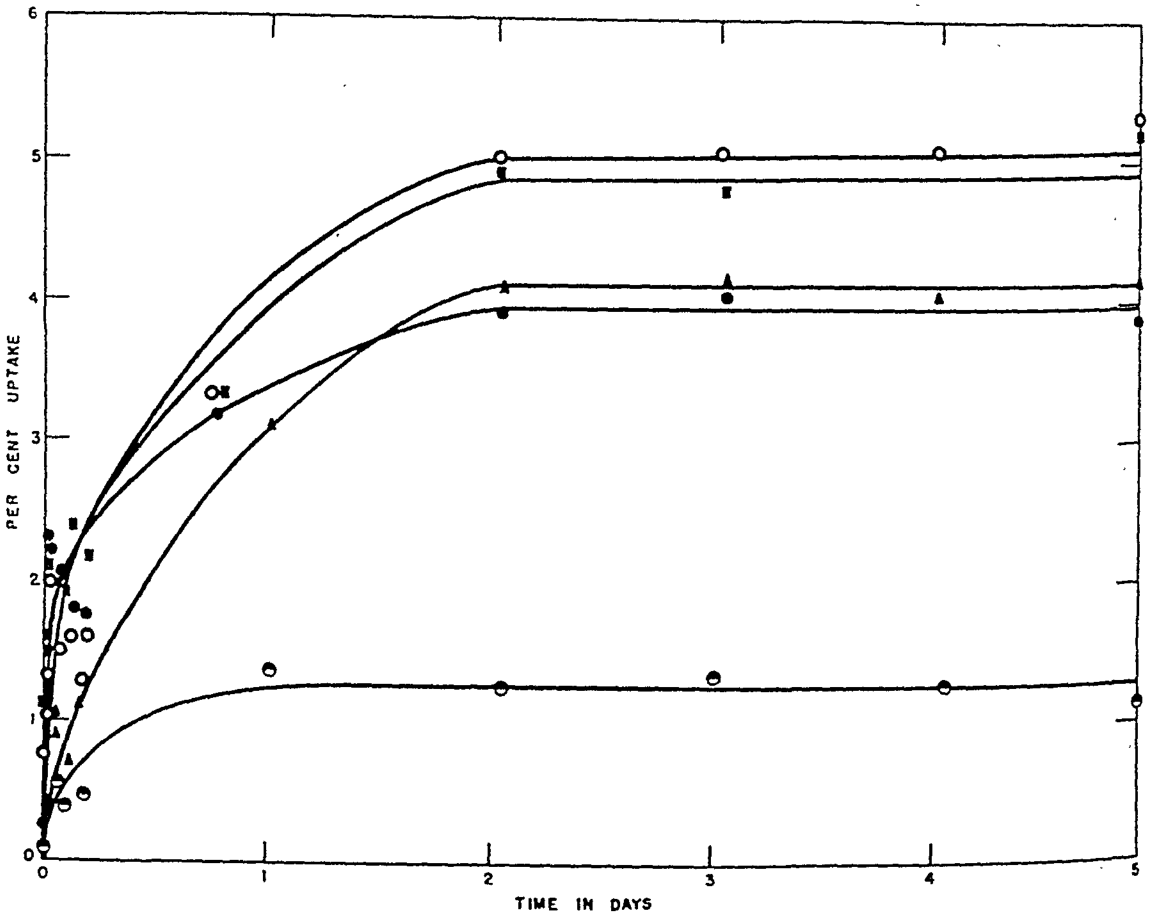

Fig. 1. Concentration of radio-iodine in the thyroids of five normal subjects.

two days following administration. The measurement of the radioactivity of the glands was continued for twenty-nine days in four subjects and for eight days in the fifth subject. After the second day of each of the five experiments, there was no further change in the form of the curves within the limits of experimental error. The estimated uptake of radio-iodine by the thyroid glands at the end of the second day ranged from 1 to 5 per cent with an average value of 3.5 per cent. The error in the measurement of radio-iodine uptake for each gland was probably less than 25 per cent.

In figure 2 are shown composite curves for the normal subjects, the hypothyroid children without goiter, the patients with non-toxic goiters, and the patients with hyperthyroidism. The single curve obtained from the child with a goiter and hypothyroidism is also included in this diagram.

The curves of radio-iodine uptake of the thyroids of the two patients with non-toxic goiters and normal basal metabolic rates were similar in form to those observed in the normal subjects. The only significant differ-

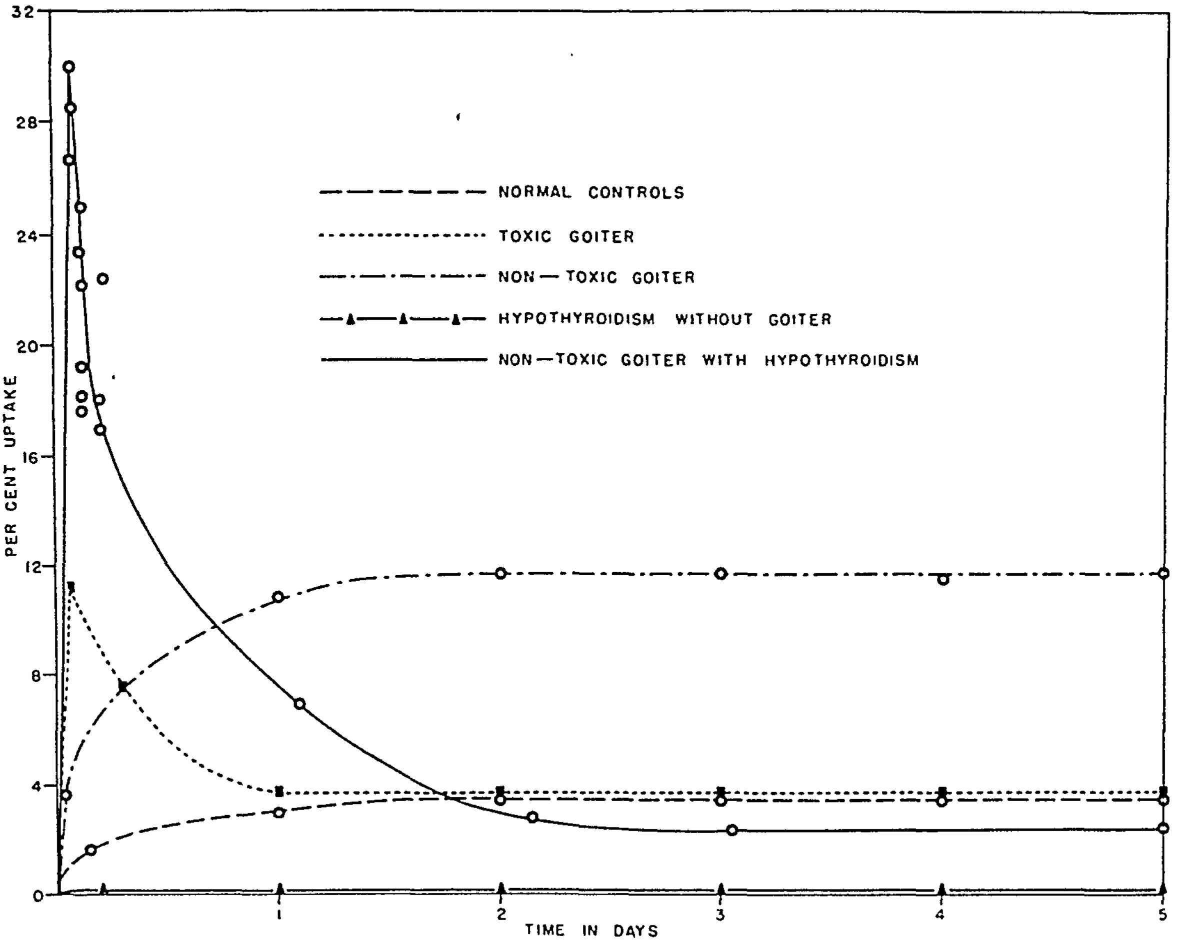

Fig. 2. Concentration of radio-iodine by the thyroids of 1, five normal subjects; 2, five adults with hyperthyroidism; 3, two adults with non-toxic goiters; 4, four children with hypothyroidism; and 5, one child with a goiter and hypothyroidism.

ence was that the percentage of radio-iodine concentrated in the thyroids of these two patients was much greater than in the normal group. The thyroids of these two patients showed no measurable change in radio-iodine content after the second day of the experiments. One patient was studied for six days and the other for two weeks. In one the uptake of radio-iodine in the thyroid at the end of the second day was 12 per cent of the administered dose, in the other it was 10 per cent.

The curves obtained from the five patients with thyrotoxicosis (fig. 2) differed strikingly in form from those of the normal group and the two adult patients with non-toxic goiter. The greatest concentration of radio-iodine in the thyroids of the thyrotoxic patients occurred at from one to four hours after its administration. Thereafter the radioactivity fell rapidly and at the end of twenty-four hours it was from one-half to one-fifth of its maximum value. The content of radio-iodine did not change significantly during the next four days. The study had to be terminated at the end of five days in two of the patients. The thyroid of the third patient lost approximately 25 per cent of the accumulated radio-iodine between the end of the second day and the tenth day, and the glands of the fourth and fifth patients showed no measurable changes in content of radio-iodine during the same period of time. The maximum uptake of radio-iodine in the thyroids of these five patients during the first day varied from 7.4 to 20 per cent. The amount of radio-iodine in the glands at the end of two days ranged from 1.5 to 7.1 per cent.

The uptake curve of the child with a goiter and hypothyroidism was similar to the curves observed in the thyrotoxic patients. The thyroid of this child attained its maximum uptake of 30 per cent of the administered radio-iodine at the end of one and one-fourth hours. Twenty-four hours later the content of radio-iodine fell to one-sixth of this value, and at the end of two days it had decreased an additional 50 per cent to 2.5 per cent of the administered amount. During the following three days it diminished approximately 10 per cent. The experiment was concluded at the end of the fifth day.

The uptake of radio-iodine by the thyroids of the four hypothyroid children without goiters ranged from 0.02 ± 0.01 per cent to 0.08 ± 0.03 per cent with an average value of 0.05 ± 0.02 per cent. These measurements are subject to considerable error because of the very small amounts of radio-iodine accumulated by the presumably atrophic thyroids of these patients.

Discussion. By the method of study we described, it was possible to follow the iodine metabolism of the thyroid gland in situ over a period of several weeks. Since the weight of iodine and the content of radio-iodine of each sample were determined before the solutions were administered and since the non-radioactive and the radioactive iodine remain in constant proportion in their passage through the body, the actual amount of iodine taken up by the thyroid gland could be calculated. For example, if a thyroid were found to contain 10 per cent of radio-iodine, then it also contained 10 per cent, or 1.4 mgm. of the 14 mgm., of the non-radioactive iodine which had been administered to the subject.

The advantages of following the uptake and discharge of iodine from the thyroid of a single subject over a period of many days are obvious.

The only other method of following iodine metabolism in various types of thyroid disorder would be to remove the thyroids in groups of subjects with thyroid diseases at varying intervals after the administration of iodine. This type of experiment is more time consuming and expensive; and, since it requires many more subjects, it is best applied to animal work.

The curves for the normal subjects and for the patients with goiter but without measurable thyroid dysfunction seem to indicate that the thyroids in both groups take up about the same amount of iodine per gram of thyroid tissue and that they retain this amount for periods of days to weeks. While the uptake curve was smooth and reached its maximum at the end of about 48 hours, the error of determination was such that fluctations within 0.3 per cent of the dose of administered iodine were obscured during the first 24 hours. On the other hand, the thyroids of the five patients with hyperthyroidism and of the one patient with a goiter and hypothyroidism took up iodine much more rapidly but were unable to retain it. It is, of course, impossible from our data to know the chemical linkage of the iodine discharged from the thyroids in the latter group.

It has been shown by pathological examination that the thyroids of patients with spontaneous myxedema are usually fibrosed and atrophic. If this were true also of the thyroids of the non-goitrous children with either severe hypothyroidism or with childhood myxedema, then the very small uptake of iodine (0.003 to 0.01 mgm.) was to be expected. Apparently the iodine concentrated in their glands was not sufficient to produce the amount of thyroid hormone required for the maintenance of a normal "thyroid" balance.

SUMMARY

The iodine metabolism of the thyroid glands of five normal subjects, five patients with hyperthyroidism, two patients with non-toxic goiters, four hypothyroid children without goiters and one patient with goiter and hypothyroidism has been studied by the use of radioactive isotopes of iodine. The uptake of iodine by the thyroids when measured over a period of five days or longer shows a characteristic curve for each of these groups. Some of the theoretical considerations which are based on the knowledge of iodine metabolism gained from this work and from the work of others have been discussed.

Appendix. In order to obtain the correct value for the radioactivity of the thyroid gland during the first two days of each experiment, a series of determinations of the radiation from the rest of the body was made by measuring the intensity of gamma radiation at the back of the neck just below the seventh cervical vertebra. The distances of this point and of the thyroid gland from the rest of the body were approximately the same. As the thickness of skin and of deeper tissues underneath the counter tube in these two positions was similar, the intensity of the gamma

radiation from the body at these two points was approximately the same. The gamma rays measured with the counter over the thyroid were the sum of the radiations from the thyroid and from the rest of the body. When the counter was against the back of the neck, the intensity of the gamma rays at this point was the sum of the gamma radiations from the body and of the small proportion of gamma rays which penetrate the neck from the thyroid.

The proportion of the gamma rays arising from the thyroid which passed through the neck was determined daily in each experiment by measuring the radioactivity in the two positions described. This proportion became constant at the end of the second day after the administration of the radio-iodine and did not change throughout the remainder of each experiment. This was due to the fact that the body radiation fell to a negligible value at the end of this time because of the rapid excretion of radio-iodine.

The quantity of radiation penetrating to the back of the neck from the thyroid gland varied from 8 to 10 per cent in the normal subjects and in the patients with small goiters. It ranged from 15 to 20 per cent in the patients with large goiters and in the one goitrous child included in this series.

The formula employed to obtain the correct value for the radioactivity of the thyroid glands during the first two days of each experiment is given below. After the second day no correction for the radiation from the rest of the body was necessary. The formula was derived in the following manner: where T_f is the measured radioactivity at the front of the neck over the thyroid, T_b is the measured radioactivity at the back of the neck, T_c is the corrected value for the radioactivity of the thyroid, R is the proportion of the radiations from the thyroid which penetrate to the back of the neck, and B represents the radioactivity arising from the rest of the body. Then:

(1). $T_f = T_c + B$ and (2). $T_b = RT_c + B$. Subtraction of (2) from (1) gives equation (3): $T_f - T_b = T_c - RT_c$. Solving for T_c, (4) $T_c = \dfrac{T_f - T_b}{1-R}$

The principal source of error in this method of computation lay in the fact that the assumed equivalence of body radiation at the thyroid and at the back of the neck was an approximation. Fortunately, during the first day of the experiments the measured intensity of radiation over the thyroid of each goitrous patient was from three to six times greater than it was over the back of the neck. This difference of intensity appeared within less than an hour after administration of the radio-iodine. Since the amount of radiation penetrating the back of the neck from the thyroid was observed to be from 8 to 20 per cent of the radioactivity of the gland, it was obvious that a large proportion of the measured radioactivity at the back of the neck arose from the thyroid. Therefore, even if the body radiation varied by as much as 50 per cent between the front and the back of the neck, the error would be relatively small for the corrected value of the radioactivity of the thyroid gland. The error in this group was estimated to be less than 10 per cent during the first day of the experiments.

The uptake of radio-iodine of the thyroid glands of the five normal subjects was considerably less during the same period of time. At the time of the last determination of the first day, the estimated quantity of radio-iodine taken up by the thyroid glands of two of this group was less than 0.7 per cent. In these two instances the error for the corrected value of the radioactivity of the thyroids was less than 50 per cent. The uptake of radio-iodine by the thyroids of the remaining three normal subjects varied from 2 to 2½ per cent during the same period of time, and the error was estimated to be less than 20 per cent.

Since most of the administered radio-iodine was excreted during the first day, it was to be expected that after the first day the error in accurate determination of the radioactivity of the thyroid glands due to radioactivity from the rest of the body would be small in both the patients and the normal subjects. Direct corroboration of this was observed in each experiment since the ratio of measured radioactivity at the back of the neck to that of the thyroid decreased, on an average, less than 5 per cent during the second day. Thereafter, as has been previously mentioned, this ratio remained constant within the limits of experimental error.

Acknowledgments. The authors wish to express their appreciation to Prof. Ernest O. Lawrence and his co-workers in the Radiation Laboratory for the preparation of the radio-iodine used in these experiments and for their advice concerning physical measurements. The suggestions and criticisms offered by Dr. John H. Lawrence of the Department of Medicine and Prof. Edwin McMillan of the Radiation Laboratory are gratefully acknowledged.

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THE EFFERENT PATHWAY FOR REFLEX PUPILLO-MOTOR ACTIVITY[1]

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Like many autonomic effectors, the iris is supplied by both divisions of the enterofective nervous system. The double innervation and the existence of autonomic antagonism affords the opportunity to study whether reciprocity exists between the centers involved in the elicitation of reflex changes in pupillary dimensions—whether, for instance, reflex dilatation is brought about by a simultaneous decrease of oculomotor tone and increase of discharge over the sympathetics.

Since Vulpian (1878) established the fact that reflex dilatation may occur in the absence of the sympathetic innervation, it has been accepted that the reflex can be brought about by third nerve inhibition. The conflicting data in the voluminous literature concern the rôle of the sympathetic system in the process. One group of investigators, e.g., Budge (1855), Luchsinger (1880), Guillebeau and Luchsinger (1882), Steil (1894), Anderson (1903) and Keller (1932) considers the participation of the smypathetic in reflex dilatation an established fact. On the other hand, Bechterew (1883), Braunstein (1894), Lieben and Kahn (1930), Bain, Irving and McSwiney (1935), and Ury and Gellhorn (1939) indicate that the sympathetic is of minor importance or else plays no part in reflexly evoked dilatation.

The following experiments were undertaken in an attempt to control for some of the possible errors in previous investigations, and to provide additional data relative to theories of the rôle of the sympathetic system in regulating the caliber of the pupil in various physiological conditions.

Methods. Cats were used exclusively. The operations performed were: 1, complete sympathectomy; 2, aseptic removal of the ciliary ganglion; 3, acute, and 4, chronic intracranial section of the oculomotor nerve; and 5, unilateral cervical sympathectomy.

Removal of the sympathico-adrenal system was accomplished in two or three stages, with aseptic precautions under ether anesthesia, according to

[1] While this paper was in press Seybold and Moore have published (J. Neurophysiol. 3: 436, 1940) the conclusion that inhibition of the oculomotor nerve is responsible for pupillary dilatation of reflex and emotional origin.

the method of Cannon, Newton, Bright, Menkin and Moore (1929). In some animals one or both cervical sympathetic trunks were excised, or unilateral or bilateral superior cervical ganglionectomy was performed. In most instances experiments on sympathectomized animals were undertaken without the use of anesthetics and after the cats had recovered from the effects of the extensive surgical intervention. In some cases the sympathectomized animals were sacrificed in an acute final experiment under urethane anesthesia.

In one animal the ciliary ganglion on one side was excised according to the technique described by Shen and Cannon (1936). The cat recovered uneventfully and was studied at different times for 2 months thereafter. It was then used in a final experiment performed under urethane.

A series of cats was prepared in which one oculomotor nerve was cut within the skull in an acute experiment. The reactions of the pupils to light, sensory stimulation, asphyxia and varying degrees of anoxia were then studied. Acute third nerve destruction was performed under nembutal, dial, urethane, or ether. Ether, when used, was maintained only during the course of the operation; after severing the oculomotor nerve the effects of the volatile anesthetic were allowed to wear off before the experiment was begun. The method of destroying the third nerve consisted of exposing the brain-stem by removal of one or both cerebral hemispheres and gently lifting the brain-stem upwards, thus uncovering the nerve as it emerged from the midbrain. It was then cut by using a long-handled sharp curved hook. Successful section was indicated by a sudden dilatation of the pupil on the operated side, while the control pupil remained unaltered. Although the operation was at first time-consuming and attended by considerable hemorrhage, with a little practice the technique was improved so that the nerve could be cut with slight blood loss in 30 minutes or less. The animals were left on a warming pad and allowed to recover from the operation for about an hour or more before any experimental procedures were instituted.

Third nerve section in cats which were to be studied over a long period of time was conducted in essentially the same manner as in the acute preparations. The anesthetic, however, was always nembutal (0.6 to 0.7 cc. per kgm. intraperitoneally), and strict asepsis was of course observed. Also, in order to have an animal with as much of the central nervous system intact as was consistent with the demands of the operation, partial hemidecortication was elected. The motor areas in these animals were intact, as it was found feasible to destroy the oculomotor nerve by removing only the occipital lobe and parts of the parietal and temporal cortices. After the successful operations the animals recovered without complications; they ate well and had regained or exceeded their original body weight by the time the experiments were started. The observations on

these animals were eventually terminated by studying the actions of various experimental procedures under anesthesia.

In anesthetized animals, adrenaline effects were routinely abolished by tying off each adrenal gland *en masse*.

Pupillary changes were usually observed, in the unanesthetized animal, by simple inspection. Occasionally animals were placed in a box, the head was held by an assistant, and the pupils were photographed under constant illumination. In anesthetized animals the pupils were sometimes photographed or were measured by placing a pair of calipers directly on the cornea. The most usual method of measuring was as follows. Indelible ink lines, 1 mm. apart, were marked on small pieces of thin cellophane with a fine ruling pen. The lids were cut and retracted and the nictitating membranes (usually relaxed) were retracted or, in some cases, excised. The strips of cellophane, then placed on each cornea, gave a direct measure of the size of the pupils.

Stimuli of different sorts were employed to study their effects on the pupils. Flashing a bright light into the eye or taking the animal into a darkened room was used to study the reactions to varying light intensities. Emotional stimulation of unanesthetized cats was supplied by confronting them with barking dogs or by producing loud and sudden noises, etc. Handling or applying weak induction shocks to the skin provided disturbing stimulation in both chronic and acute experiments. When varying degrees of anoxia were required the tracheal cannula was connected with a Tissot spirometer which contained low-oxygen mixtures. Asphyxiation resulted from attaching a rubber bladder to the tracheal cannula, thus forcing the animal to rebreathe its expired air. The central stump of the bared sciatic nerve was used for afferent stimulation; shielded silver-wire electrodes were placed on the trunk and the stimulating current was derived either from a Harvard inductorium (faradic shocks) or from a multivibrator (condenser discharges of varying frequencies and intensities).

The drugs employed in these experiments were eserine salicylate (Sharp and Dohme), acetylcholine chloride (Merck), pilocarpine nitrate (Merck), and adrenalin chloride (Parke-Davis).

RESULTS. 1. *Completely sympathectomized cats.* In such animals the pupil is small. That this constrictor tone may be suppressed has been abundantly confirmed since the early work of Vulpian (1878). That the adrenal glands are not required for the appearance of pupillary dilatation was demonstrated by Bain, Irvin and McSwiney (1935). The possible action of other humoral agents, however, has not been hitherto excluded. Sympathectomy offers a certain method of obviating sympathetic humoral effects, since the release of adrenaline or sympathin into the blood stream is no longer possible.

A few sympathectomized cats were prepared especially to study the

reactions of the iris, but most of the observations were made on cats which were used for other experiments and had been deprived of their sympathico-adrenal systems 2 weeks to 3 months previously. At least 25 such animals were examined. In all cases, struggle, excitement, or sensory stimulation, produced by a number of methods, was attended by prompt pupillary dilatation. The same result followed whether or not the cervical sympathetic trunks or superior cervical ganglia had been previously excised. No contraction of the nictitating membrane occurred with the same types of stimulation. When the sympathectomized animals were sacrificed in a final experiment under anesthesia, faradization of the central end of the bared sciatic nerve elicited rapid and marked dilatation of the pupil, but had no action on the nictitating membrane.

B. *Cat with unilateral excision of the ciliary ganglion.* As is well known, removal of the ciliary ganglion leads to a widely dilated pupil. The observation of Anderson (1903) and of Shen and Cannon (1936), that the light reflex is absent in the parasympathectomized iris, was readily confirmed. In addition, I thought that by inducing an even more dilated pupil than was already present—e.g., by the injection of adrenaline—central inhibition of the sympathetic might be unmasked. Even with such a wide pupil exhibition of an intense light elicited no constriction. The converse of this experiment also failed to point to sympathetic participation in the light reflex: both eyes were treated with pilocarpine; when maximal myosis had occurred the cat was placed in a dark room for 20 minutes. The pupil on the normal side dilated under these conditions, while the parasympathetically denervated iris was unaffected by the dim light, and the pupil remained quite narrow.

Emotion or afferent stimulation caused no widening on the side deprived of its oculomotor nerve, although the usual dilatation took place on the intact side. Even after the local instillation of myotic drugs had narrowed the pupils, stimuli of various sorts evoked no dilatation of the denervated side, although they caused marked and prompt expansion of the pupil on the normal side. The drugs employed did not alter the reactivity of the pupil, since direct stimulation of the sympathetic trunk in the neck elicited rapid dilatation.

It is interesting to note that in some animals *both* nicitating membranes were withdrawn on afferent stimulation, while the same stimulation caused retraction of the iris only when the third nerve was intact. This is in agreement with an observation of Karplus and Kreidl (1918). On the other hand, Keller (1932) concludes that manipulation of the midbrain animal causes increased sympathetic activity (retraction of the nictitating membrane, separation of the lids and dilatation of the pupil) since these reactions are not present on the sympathectomized side. But in the protocols of his cats 225 and 226 I find no indication that the *irises* on the two

sides behaved differently, although there is an undoubted difference between the activity of the membranes and eyelids on the two sides. This absence of parallelism between pupillary and other ocular effects should point out the obvious fallacy of inferring a sympathetic effect on the iris simply because it has such an action on the nictitating membrane.

The following excerpts from the protocol of this cat indicate the salient features of the results.

Protocol of cat 1—♂, 3.4 kgm.

11/22/38. Left ciliary ganglion excised aseptically under ether anesthesia.

11/24/38. Left pupil wide and insensitive to light. Right pupil normal.

12/ 5/38. No reflex to bright light on left side has been obtained since operation.

12/ 6/38. 0.5 mgm. adrenaline injected subcutaneously. Left pupil widened slightly, right dilated markedly. Bright light flashed into eyes. Right pupil constricted rapidly, left unaffected.

1/ 3/39. 2:25 p.m.—1 drop 0.4 per cent pilocarpine nitrate instilled in each conjunctival sac.

2:40 p.m.—Both pupils constricted to slits.

2:40–3:00—Cat in dark room. Right pupil wider than before being taken into room; left remained the same as at 2:40.

1/ 5/39. 9:38 a.m.—2 drops 1 per cent eserine salicylate in each eye.

9:50 a.m.—Pupils narrow. Left smaller than right.

10:05 a.m.—Dog brought into room. Cat excited. Right pupil widened when dog was noticed, left pupil unaffected.

1/22/39. Acute experiment. Urethane. Adrenals tied. Cellophane markers on each cornea.

12:25 p.m.—Left pupil 14.0 mm., right 3.5 mm. Both nictitating membranes relaxed.

12:47 p.m.—2 drops 1 per cent eserine instilled in each eye.

12:59 p.m.—3 drops 0.1 per cent acetylcholine put in each conjunctival sac.

1:38 p.m.—Left pupil 2.3 mm., right 3.0 mm.

1:40 p.m.—Stimulation of central end of left sciatic nerve (1.5 volts, secondary at 8.5 cm.). No dilatation of left pupil; marked expansion on right side. Contraction of *both* nictitating membranes.

2:00 p.m.—10 γ adrenaline injected intravenously. Dilatation of both pupils and retraction of both nictitating membranes.

2:20 p.m.—Stimulation of cephalic end of cut left cervical sympathetic trunk (1.5 volts, 12 cm.). Sudden widening of pupil and contraction of membrane.

3. *Cats with acute third nerve section.* Ten cats were studied about an hour after the oculomotor nerve had been cut within the skull. The adrenal glands were routinely tied off; in most experiments the cervical sympathetic trunk was severed on the side opposite to the third nerve destruction.

The widely dilated pupil is insensitive to light. The loss of the light reflex is due solely to absence of the oculomotor nerve and not to other effects of the operation, e.g., removal of the cortex "shock," etc., since in

animals deprived of both hemispheres the iris with oculomotor nerve intact reacts normally to variations in the light intensity.

Afferent impulses—whether set up by direct electrical stimulation of the bared sciatic nerve, by pinching, pricking or faradizing the skin with induction shocks—never produced pupillary dilatation on the parasympathectomized side. Constriction of the wide pupil was frequently brought about by the use of myotic drugs, applied locally, but this procedure also failed to disclose dilatation of the pupil on afferent stimulation. "Shock" cannot explain these negative findings; for on the side with the third nerve supply intact the pupil dilated readily under the same conditions. Also, when the sympathetic was cut on the side opposite that on which the third nerve had been removed, stimulation of the sciatic caused the pupil on the sympathectomized side to dilate, while the opposite pupil remained fixed.

In 3 animals, in which the third nerve on one side was cut and both sympathetics were intact, sciatic stimulation elicited dilatation of only the normal pupil but retraction of *both* nictitating membranes (see p. 147).

Although the parasympathectomized iris could not be reflexly excited to produce expansion of the pupil, injection of adrenaline occasioned a further dilatation of the wide pupil. Also, direct stimulation of the cervical sympathetic trunk with weak induction shocks caused widening whether or not a myotic drug had been previously administered.

The following abbreviated protocol of cat 2 will serve to indicate the reactions of the pupils of a cat subjected to destruction of the oculomotor nerve on one side.

Protocol of cat 2—♂, 2.9 kgm.—3/9/39.

Urethane, adrenals tied. Right sciatic exposed and prepared for central stimulation. Cellophane marker on each cornea.

2:55 p.m.—Decortication and section of left third nerve completed.

4:07 p.m.—Right sciatic stimulated centrally (1.5 volts, 6 cm.). Rapid widening of right pupil; left pupil (11.0 mm.) unchanged. Retraction of both nictitating membranes.

4:12 p.m.—5 drops 0.1 per cent eserine in each conjunctival sac.

4:20 p.m.—2 drops 0.04 per cent acetylcholine in each eye.

4:52 p.m.—Left pupil, 3.5 mm., right, 4.0 mm.

4:58 p.m.—Stimulation of right sciatic. Right pupil 6.5 mm.; left remained at 3.5 mm.

5:12 p.m.—Stimulation of cephalic end of cut left cervical sympathetic nerve (1.5 volts, 12 cm.) produced rapid and wide dilatation.

The effects of low-oxygen tension and asphyxia were studied on some animals with one third nerve removed acutely. The results were not clear, since usually no change was observed until the animals were moribund. Generally, 5 to 7 per cent oxygen mixtures or asphyxiation caused, after 2 to 6 minutes, dilatation of both the normal and denervated sides. Dilatation occurred during oxygen lack also when the sympathetic had been

severed previously. One adrenalectomized animal, lacking the oculomotor nerve on the left and the cervical sympathetic on the right side, was asphyxiated almost to death. Both pupils dilated, the right widening more obviously from its resting position than the left. Asphyxiation was discontinued, and the animal recovered. Then the left sympathetic trunk was cut in the neck, thus leaving a completely denervated iris on this side. Asphyxiation was begun again and once more elicited widening of both pupils. The dilatation occurred slowly and synchronously on both sides. This experiment indicates that dilatation in this case was not of neural origin. It might be referred to some direct effects on the iris, due to asphyxia.

4. *Cats with chronic oculomotor nerve section.* In order to rule out beyond doubt the interference of "shock" and also to be able to dispense with anesthetics, intracranial third nerve section was performed in 3 cats which were then allowed to recover for at least 2 weeks before being used again. These animals exhibited pupils which were unresponsive to light, whether or not pupillo-constrictor or dilator drugs had been employed. In none of these animals did stimulation of the skin or excitement cause dilatation on the parasympathectomized side. Likewise, in the final experiment under urethane, sciatic stimulation failed to indicate any participation of the sympathetic centers in the process of reflex dilatation.

A protocol of one experiment on cat 3 exemplifies the typical data obtained in several observations on the 3 animals.

Protocol of cat 3—♀, 2.2 kgm.

6/ 2/39. Right third nerve cut within skull under nembutal. Motor areas intact.

6/ 5/39. Cat well; eating of own accord. Right pupil wide and fixed to light. Left side has normal reactions to light.

6/31/39. Cat has recovered from operation and is eating well. Right pupil wide and insensitive to bright or dim light.

6/22/39. 11:07 a.m.—One drop 1 per cent eserine in each conjunctival sac.
11:30 a.m.—One drop 1 per cent acetylcholine in each eye.
11:40 a.m.—Both pupils narrow; left slightly larger than right.
12:00—Cat put in box and became excited. Left pupil widened rapidly; right unaffected. Other stimuli—pinching the pinna, pricking the skin, pulling whiskers, stimulating the skin with weak induction shocks, and confronting it with a barking dog—also caused the left pupil to dilate, but the right side remained unaltered.

5. *Cats subjected to unilateral cervical sympathectomy.* A final series of experiments was devised to test for a possible participation of the sympathetic outflow in reflex pupillary dilatation. An attempt was made, after tying off the adrenal glands and cutting one sympathetic trunk in the neck, to find a stimulus which would induce reflex expansion of the pupil on the normal side without affecting the denervated side. To this end varying strengths and frequencies of condenser shocks were applied to the bared

sciatic nerve and both eyes were observed for a possible differential effect of the stimulation. In addition, the animals were subjected to varying degrees of asphyxia in order to determine whether any differences would appear on the two sides.

All the foregoing experiments were negative so far as the activation of sympathetic centers in pupillary dilatation is concerned. Thus, if a series of shocks delivered to the sciatic nerve brought about an increase in the caliber of the normal pupil, dilatation also occurred on the denervated side. The dilatation began at the same time on both sides and both pupils ran a parallel course during and after stimulation. If the frequency of the shocks was too slow, no effect was seen on *either* side. When a stimulus was of insufficient intensity to cause a response on the denervated side, it also failed to elicit any change in the intact eye. As the strength of current was gradually increased, a point was reached at which both pupils dilated. No stimulus ever caused one pupil to widen without producing a corresponding effect on the other. Hence, no differential action could be observed by varying either the frequency or strength of the shocks applied to the sciatic nerve.

Differing degrees of asphyxia likewise failed to cause dilatation of one pupil without similarly affecting the opposite side. Deepening the narcosis did not alter either the effects of nerve excitation or asphyxia. The pupils in all cases began to dilate at the same time, remained wide concurrently, and constricted simultaneously when the stimulus was removed.

The sympathectomized side invariably presented a pupil which was narrower than the normal. Also, when the stimulus was acting, the pupil on the denervated side dilated less markedly than the control. This fact does not require the postulation of an increase of sympathetic discharge. It may be explained on the basis of an alteration of the normal sympathetic-parasympathetic balance. If a constant tonic activity by way of the cervical sympathetics is assumed, then a decrease of constrictor activity would lead to dilatation by two means: 1, a direct effect produced by the relaxation of the *sphincter pupillae*; and 2, a relative release of the dilator muscle from the balancing force of the opposed constrictor muscle. Since the second method is abolished when the sympathetic is destroyed, it is probable that the resultant widening of the pupil will be less extreme than when both mechanisms are utilized.

The following protocols are typical of 6 similar experiences which indicate that the sympathetic system plays no significant rôle in dilatation under the conditions investigated.

Protocol of cat 4—♀, 2.2 kgm.—3/23/39.
Urethane. Adrenals tied. Right cervical sympathetic cut. Left sciatic prepared for central excitation. Cellophane marker on each cornea.

4:15 p.m.—Right pupil, 0.3 mm.; left, 1.3 mm. 4 minutes of stimulation of central

sciatic by condenser shocks (1 per sec., 2 volts) caused no change in either pupil.

4:21 p.m.—Right pupil, 0.3 mm.; left, 1.3 mm. After 2 minutes of stimulation (1 per sec., 4 volts) the right pupil was 1.0 mm., the left 2.6 mm. Both pupils began to dilate at the same time, reached the maximum width together, and returned to their original sizes together when the stimulation was halted.

4:37 p.m.—Right pupil, 0.3 mm., left, 1.0 mm; 2.5 minutes of stimulation (1 per sec., 1 volt) caused no change on either side. After 1 minute of stimulation at 4 per sec. and 1 volt, the right pupil was 1.0 mm., the left, 3.0 mm.

5:19 p.m.—Right pupil a slit; left, 1.5 mm. Stimulation at 15 per sec., 2 volts. After 30 sec. right was 2.0 mm., left was 3.5 mm. After 2 minutes of continuous stimulation, right pupil was 0.9 mm., left was 2.7 mm.

5:30 p.m.—Anesthesia deepened by injecting more urethane.

5:30-6:20—Series of intensity and frequency variations employed as before. Both pupils change together or not at all.

6:40 p.m.—Right pupil, 0.7 mm.; left, 3.0 mm. Tetanic stimulation, 4 volts. After 30 sec., right was 2.0 mm., left, 5.0 mm. After 1 minute of continuous excitation, right was 1.0 mm., left, 4.0 mm. After 2 minutes, right was 1.0 mm., left, 4.0 mm.

Protocol of cat 5—♂, 3.4 kgm.—5/16/39.

Dial. Adrenals tied. Right cervical sympathetic cut. Cellophane marker on each cornea.

4:20 p.m.—Right pupil, 1.5 mm., left, 3.0 mm. Empty rubber bladder attached to tracheal cannula. Both pupils began to dilate 35 sec. after asphyxiation was started. After 1 minute, right pupil was 2.0 mm., left, 6.0 mm. After bag was removed both pupils narrowed rapidly to original caliber.

Discussion. The material presented above points to the conclusion that reflex pupillary motor effects are mediated solely by the oculomotor nerve. Anderson's (1903) unsuccessful attempt to show a sympathetic component in the light reflex has been confirmed (p. 148). Gullberg, Olmsted and Wagman (1938), on the contrary, concluded that, in the rabbit, the sympathetic took part in the dilatation of dark-adaptation, since the widening was greater and perhaps more rapid on the normal than on the sympathectomized side. As has been stated (p. 151), this fact does not demand the positing of an increased sympathetic activity for its explanation. Gullberg, Olmsted and Wagman interpret the break in the curve (see their fig. 3) as being caused by the sympathetic acting by itself. Even if this assumption is valid, from their figure it is apparent that the change in the components causing dilatation occurs at approximately 100 seconds and not at 20 seconds as these authors state.

That constriction of the pupil is accomplished by the third nerve alone is accepted by all writers. Six main avenues of investigation have led to the hypothesis that when the pupil dilates in various physiological con-

ditions (emotion, asphyxia, afferent stimulation, etc.) the effect is mediated by increased sympathetic activity: 1, the demonstration by Bernard, Budge, and others that section of the cervical sympathetic resulted in pupillary constriction; 2, the work of Bernard (1852) which showed that direct stimulation of this nerve caused expansion of the pupil; 3, the researches of Karplus and Kreidl (1909) in which direct stimulation of the diencephalon evoked dilatation only if the sympathetic was intact; 4, Budge's (1855) stimulation of the dorsal roots of the isolated cord which elicited widening of the pupil; 5, the experiments of Luchsinger (1880) and Guillebeau and Luchsinger (1882) who, after transecting the cord below the medulla and cutting the sympathetic on one side, found that afferent stimulation brought about dilatation on the normal, but not on the sympathectomized side; and 6, the studies of Anderson (1903) which indicated that pupillary dilatation occurred during afferent stimulation even after the third nerve was destroyed.

The data presented in this paper are incompatible with the concept that sympathetic nervous mechanisms play any rôle in the production of reflex changes in the size of the pupil. The reasons why the above authors have reached an opposite interpretation of the part of the sympathetic may now be considered.

The studies mentioned in (1) and (2) above need not be considered, for they did not deal with *reflex* alterations. Also, the work of Karplus and Kreidl in no way forces one to accept reflex sympathetic pupillary effects, for descending fiber tracts from the diencephalon, which are not activated in dilatation of reflex origin, may have been stimulated.

When Budge (1855) isolated the section of the cord sending fibers to the iris from the rest of the cerebrospinal axis and found that stimulation of the dorsal roots led to prompt widening of the pupil, he considered this to be proof that the ciliospinal center had been reflexly aroused to action. An escape of current to the nearby ventral roots might have occurred to invalidate Budge's conclusions.

Luchsinger's (1880) statement that dilatation of the pupil resulted from afferent stimulation if the cord was cut above the ciliospinal center (but not after cervical sympathectomy in the spinal animal) has been refuted by Braunstein (1894) and by Bain, Irving and McSwiney (1935). Luchsinger employed strychnine, cocaine and other drugs, and it is possible that the discrepancy was due to the use of these pharmacological agents.

Anderson's (1903) report that sciatic stimulation or handling of the animal causes dilatation in the absence of the third cranial nerve cannot be accepted, for evidence against it has been reported by Bain, Irving and McSwiney (1935), Ury and Gellhorn (1939), and it is contradicted by the present experiments. Ury and Gellhorn suggested that Anderson utilized deep chloroform anesthesia and thus "functionally eliminated all higher

centers and created (by disinhibition) a state of heightened excitability of the ciliospinal center." Since Anderson mentions some dilatations of slow onset, it is also possible that humoral agents (adrenaline, sympathin) were not excluded.

Significant experimental data which have accumulated in the last decade—and which support the careful studies of Bechterew (1883) and particularly of Braunstein (1894)—make it increasingly apparent that reflex pupillary dilatation depends largely, if not completely, on depression of oculomotor tone. Lieben and Kahn (1930), on the basis of ablation experiments, attributed the mydriasis of emotional states to third nerve inhibition alone. Bain, Irving and McSwiney studied the effects of central splanchnic excitation on the size of the pupil; they eliminated the adrenal glands and also showed that large blood-pressure changes were not responsible for the ocular effects observed. Section of the third nerve or transection of the cord in the cervical region rendered the irises unresponsive to stimulation of the splanchnic nerves. Ury and Gellhorn urged that third nerve inhibition be accepted as the mechanism responsible for dilatation due to pain. They stated that only when the excitability of the ciliospinal center is raised (e.g., by metrazol) does the sympathetic add any component to the dilatation.

The present data add further support to the evidence of recent investigators who deny the contribution of the sympathetic in the process. This statement does not deny the importance of sympathetic humoral agents; indeed, the sympathetic humoral and the parasympathetic neural mechanisms coöperate to cause pupillary dilatation.

SUMMARY

1. Completely sympathectomized cats exhibit a normal light reflex. Physiological stimuli evoke pupillary dilatation which is indistinguishable from that of the normal animal (p. 146).

2. Unilateral excision of the ciliary ganglion, or acute or chronic destruction of the oculomotor nerve abolishes the light reflex. These operations remove the ability of the pupil to dilate to emotional, painful, or afferent nerve stimulation (pp. 147, 148 and 150; protocols of cats 1, 2 and 3).

3. Anoxia or asphyxia may cause dilatation on the parasympathectomized side, but this effect is considered to be due to other than sympathetic nervous mechanisms (p. 150).

4. No differences were established between a normal and sympathectomized eye by the use of a variety of frequencies and intensities of electrical stimulation of the sciatic nerve, or by asphyxiation of different degrees of severity (p. 151 and protocols of cats 4 and 5).

5. It has been concluded that, in the cat, under the conditions investi-

gated, no significant part in pupillary dilatation of reflex origin is played by the sympathetic nervous mechanisms.

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THE RELATION BETWEEN BIREFRINGENCE AND CONTRACTILE POWER OF NORMAL, HYPERTROPHIED, AND ATROPHIED SKELETAL MUSCLE

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All modern theories of muscular contraction take in account the submicroscopic crystalline arrangement of the muscle proteins as revealed by x-ray spectrography or birefringence observations (Fischer, 1936, 1940). If the crystalline structure really does play an important part in the contractile process, there should exist a certain parallelism between contractile power and the degree of completeness of the crystalline arrangement. The birefringence of a muscle is dependent to a large extent on this completeness of the crystalline pattern. The few birefringence data available in the literature for muscle of various species cannot be evaluated for this purpose. The contractile forces of these various muscles have not yet been determined accurately, and, what is much more important, birefringence analysis has revealed that the submicroscopical patterns of muscles of various species are not identical enough to permit the use of the data on total birefringence as a measure of the completeness of their crystalline arrangements (Fischer, 1940).

In an attempt to secure figures permitting such a comparison, the maximal isometric forces and the total birefringence of gastrocnemius-soleus muscles of rats were measured. Since the natural variations of the contractile power (tension per gram weight) and the birefringence of gastrocnemii from different rats are rather small, such a comparison is only possible on a very capacious experimental material; and even then the relations between strength and birefringence can be established only for a very narrow range. The cumbersome method needed for the determination of the average birefringence value of one muscle limits furthermore such a statistical investigation. Therefore, in this paper the main emphasis is laid upon the comparison of matching muscle pairs, one of them being normal, while the contractile power of the other has been either increased by previous training or decreased in consequence of denervation atrophy.

Material and method. All experiments are performed on rats 4 to 8 months old. The first series was on normal rats to find out the normal range of variations between matching gastrocnemii. In the second series, one hind leg of the rats had been stimulated twice daily for about twenty

minutes by faradic currents to produce a hypertrophy of the muscles of that side. By a motor-driven mechanical device the currents of an induction coil were turned on and off each half second, thus producing 60 short but strong contractions per minute. After one to two minutes of stimulation a rest period of about the same duration was interpolated and the direction of the currents changed. Thus each training period of 20 minutes included about 10 to 11 minutes of actual stimulation. Special care was taken to use just maximal stimuli. The animals were fastened to a rat-board and were kept under very light ether narcosis during actual stimulation. In the third series of experiments, one sciatic nerve had been resected aseptically for a length of 1 cm. at least several days or weeks previous to the final experiment.

Tension measurement. Under light ether anesthesia, both knee joints were fixed rigidly by clamps, the Achilles tendons exposed, then cut, and connected with a twin pair of isometric tension wire levers. The muscles were stimulated directly with repeated electrical shocks, optimal for strength, duration and frequency (Fischer, 1939), and the maximal tetanic tension was recorded on a smoked drum. After the tension measurement, anesthesia was interrupted and the rat released from its fixation. At least two hours were allowed for restoration of more or less normal blood supply in the stimulated muscles before the rat was killed by a blow on the head.

Weight and dry substance determination. Theoretically it would be correct to relate the total strength of a muscle to its physiological cross section (Fischer and Steinhausen, 1925). However, a determination of the physiological cross section of the gastrocnemius-soleus of the rat is rather impractical. Therefore, the tension per weight unit was chosen as a measure of the contractile power. Both muscles were dissected carefully and cleaned of other tissues, and the tendons were cut in exactly corresponding places. The muscles were weighed immediately and three to four muscle fiber bundles were cut out for the birefringence determination. Then the muscles were weighed again and dried at 108°C. to a constant weight.

Birefringence determination. The three to four small bundles of muscle fibers taken from each gastrocnemius (from corresponding parts of the matching muscles) were put in watch glasses with oxygenated buffered Tyrode solution. The bundles were then carefully teased into thinner bundles consisting of two or three fibers. The double refraction of these small fiber bundles was determined as phase differences under the polarization microscope by means of a graduated quartz wedge. The thickness of the bundles was measured by the use of the micrometer screw of the microscope. The latter measurement was facilitated by added traces of India ink to the Tyrode solution. The phase difference observed

was divided by the measured thickness of the bundles in order to find the birefringence per millimeter thickness. From the various values obtained for the 18 to 22 fiber bundles of a single muscle, the average value for the birefringence of that muscle was computed. The birefringence mentioned in this paper refers always to what is known as "total birefringence" since no attempt has been made to analyze the measured birefringence concerning its two components: "crystalline birefringence" and "form birefringence."

Results. The first series, that on normal muscles (table 1), was used mainly to establish the normal variations, which must be expected between left and right gastrocnemii. The maximal variation in wet or dry weight

TABLE 1

Comparison of left normal with right normal gastrocnemius in rats

NO.	WET WEIGHT		DRY WEIGHT		TENSION		GRAM TENSION PER GRAM WET WEIGHT	GRAM TENSION PER GRAM DRY WEIGHT	TOTAL BIREFRINGENCE	
	Right	Left	Right	Left	Right	Left	Left in % of right		Right 10^{-3}	Left in % of right
	grams	*grams*	*gram*	*gram*	*grams*	*grams*				
1	2.049	2.067	0.468	0.468	1700	1750	102.0	102.2	2.38	101.3
2	1.768	1.732	0.393	0.395	1950	1950	103.7	99.6	2.59	99.6
3	2.017	2.038	0.473	0.478	1450	1500	102.1	102.3	2.29	97.6
4	2.266	2.331	0.548	0.557	1800	1850	99.7	101.3	2.33	100.9
5	1.952	1.927	0.457	0.452	1700	1650	98.5	98.4	2.27	100.0
6	2.080	2.102	0.494	0.492	1950	1950	99.1	100.7	2.38	99.3
7	2.712	2.749	0.623	0.647	2350	2450	103.1	100.4	2.34	98.6
8	2.271	2.285	0.542	0.549	2150	2050	94.8	94.2	2.26	101.0
9	2.141	2.171	0.509	0.514	1900	1950	101.4	101.1	2.35	101.4
10	2.100	2.085	0.502	0.496	1850	1850	100.8	101.2	2.40	99.6

observed for matching normal muscles is 2.9 per cent. The maximal difference for tension per gram weight reaches 5.2 per cent for wet weight and 5.8 per cent for dry weight. The maximal birefringence difference is 2.4 per cent. The average variations are much less, and table 1 indicates clearly that contractile power as well as birefringence of matching muscles can be regarded as practically equal.

In the second series, that with muscle hypertrophy produced by electrical training, some of the rats responded poorly to the training. However, for the problem in which we are interested in this paper, it is only of importance that hypertrophy could be produced at least in a large number of rats. For the sake of clearness table 2 includes only those animals in which the increase in weight of the trained muscle was at least twice as large as the maximal weight variation observed between pairs of normal

muscles. The observed tension increase of the trained muscles corresponds to their weight increase. In consequence the values of tension per grâm weight are practically unaltered by the training. Also the birefringence of the trained muscles is the same as that of their untrained partners.

In the third series, comparing atrophying muscles with their normal partners (table 3), somewhat similar results were obtained as long as the atrophy did not become too severe. No appreciable diminution in birefringence or in tension per gram weight (the latter showing even the tendency to increase) occurs during the first 9 to 11 days, although the weight loss and loss in total tension proceed to about 30 per cent at the end of that

TABLE 2

Comparison of right trained with left normal gastrocnemius in rats

DAYS IN TRAINING	WET WEIGHT		DRY WEIGHT		TENSION		GRAM TENSION PER GRAM WET WEIGHT	GRAM TENSION PER GRAM DRY WEIGHT	TOTAL BIREFRINGENCE	
	Left	Right	Left	Right	Left	Right	Right in % of left		Left 10^{-3}	Right in % of left
	grams	*grams*	*gram*	*gram*	*grams*	*grams*				
5	1.560	1.670	0.374	0.415	1550	1650	99.4	96.2	2.53	101.4
10	2.045	2.240	0.488	0.528	2350	2550	99.1	100.3	2.60	98.5
12	1.617	1.883	0.399	0.465	1800	2150	102.4	102.7	2.47	99.5
13	1.529	1.692	0.365	0.407	1450	1600	99.4	98.8	2.35	98.0
14	2.059	2.250	0.443	0.486	1600	1750	99.9	99.6	2.28	99.1
16	2.376	2.606	0.577	0.625	2450	2750	102.1	103.6	2.57	98.8
17	2.015	2.277	0.472	0.536	1750	2000	101.2	100.8	2.22	99.1
18	1.754	1.939	0.417	0.469	1450	1600	99.8	98.1	2.26	100.9
18	1.541	1.775	0.362	0.423	1650	1900	100.2	98.7	2.43	98.3
25	1.806	2.048	0.436	0.494	1700	1950	101.1	100.0	2.38	101.4
41	2.010	2.349	0.450	0.523	1950	2300	101.0	101.4	2.50	100.9
47	1.971	2.293	0.445	0.517	1750	2100	103.4	103.4	2.45	98.9
54	1.785	2.187	0.443	0.537	1700	2100	100.2	101.8	2.50	99.2

period. However, in the later phase of atrophy, both tension per gram weight and birefringence drop considerably, but approximately at the same rate. Thus, for the second stage of atrophy, the loss in completeness of crystalline structure of the muscle substances corresponds roughly to the loss in contractile power.

Figure 1 represents graphically the absolute values for birefringence and tension per gram fresh weight of all normal muscles of the three series (dots), of the trained muscles (crosses), and of the denervated muscles (circles). Since training the muscles neither changed their birefringence nor their specific strength (table 2), the values for these muscles can be regarded also as values for normal muscles. It is obvious from the graph

that for normal muscles there exists statistically a relation between birefringence value and contractile power, the latter increasing with an increase of the former. Since the number of observations is limited and the range of natural variations is small, this relation between birefringence and

TABLE 3

Comparison of left atrophied with right normal gastrocnemius in rats

DAYS OF ATROPHY	WET WEIGHT		DRY WEIGHT		TENSION		GRAM TENSION PER GRAM WET WEIGHT	GRAM TENSION PER GRAM DRY WEIGHT	TOTAL BIREFRINGENCE	
	Right	Left	Right	Left	Right	Left	Left in % of right		Right 10^{-3}	Left in % of right
	grams	*grams*	*gram*	*gram*	*grams*	*grams*				
3	2.226	1.973	0.511	0.454	2050	1850	102.0	101.6	2.45	98.4
5	2.524	2.218	0.610	0.523	1950	1700	99.6	101.7	2.23	99.6
6	1.815	1.522	0.428	0.359	1800	1500	99.4	99.3	2.42	101.2
7	2.091	1.689	0.494	0.395	2150	1800	103.8	104.7	2.49	99.1
8	1.877	1.421	0.456	0.334	1750	1300	98.1	101.8	2.50	99.5
9	1.861	1.432	0.450	0.327	1850	1450	101.9	107.9	2.45	101.2
9	2.929	2.060	0.673	0.464	2350	1800	109.1	111.1	2.26	101.8
10	1.971	1.109	0.444	0.242	2350	1350	104.2	105.1	2.61	97.6
10	1.619	1.161	0.397	0.270	1950	1300	92.6	98.2	2.54	96.9
11	2.381	1.615	0.561	0.363	2450	1700	102.7	107.2	2.48	97.5
11	2.085	1.308	0.495	0.295	2150	1300	96.5	101.3	2.44	93.6
11	1.804	1.256	0.422	0.276	1850	1350	104.9	106.1	2.36	98.7
11	2.455	1.635	0.589	0.360	2550	1700	100.0	109.1	2.46	98.4
12	1.438	0.550	0.331	0.130	1450	450	81.4	78.9	2.48	80.6
12	2.650	1.703	0.622	0.382	2350	1450	96.1	100.5	2.38	94.0
13	2.474	1.380	0.575	0.309	2250	1050	83.7	86.9	2.32	83.7
15	2.287	1.204	0.525	0.275	2150	850	75.0	75.6	2.44	75.9
16	1.543	0.590	0.364	0.131	1450	450	80.3	86.2	2.37	82.4
17	1.985	0.893	0.464	0.193	1950	700	80.0	85.7	2.30	78.7
19	1.537	0.832	0.362	0.182	1500	650	80.0	86.1	2.54	74.1
20	1.854	0.842	0.437	0.194	1850	500	59.5	60.9	2.44	67.2
21	1.744	0.626	0.389	0.132	2100	600	79.5	84.0	2.58	71.4
22	1.609	0.595	0.375	0.136	1950	600	82.9	84.7	2.63	65.4
23	2.056	0.804	0.484	0.175	2100	550	67.0	72.7	2.43	71.3
26	1.226	0.529	0.278	0.113	1200	350	67.8	71.6	2.45	56.1
29	1.906	0.621	0.464	0.141	2150	500	70.5	80.0	2.47	67.0
33	1.793	0.561	0.416	0.117	1850	350	59.9	67.4	2.34	63.3

tension per gram weight could be represented by a straight line as well as by the curve drawn in figure 1. The latter is a logarithmic curve and corresponds to the function: birefringence = k × log. contractile power. The values for atrophying muscles do not correspond to this equation. For the muscles in the first stage of atrophy, the values fall in the range for

normal muscles. However, during the second stage of atrophy, the loss in contractile power is less than one ought expect according to the relation between birefringence and contractile strength in normal muscles. In the second stage of atrophy, birefringence changes more or less proportionally to the contractile power, and not to the latter's logarithm.

That in the first stage of atrophy, despite a weight loss up to 30 per cent, no change in birefringence nor in contractile power occurs, indicates that the weight loss during atrophy is a more or less independent process, not directly connected with the possible degenerative changes resulting in destruction of the submicroscopical structure and diminution of con-

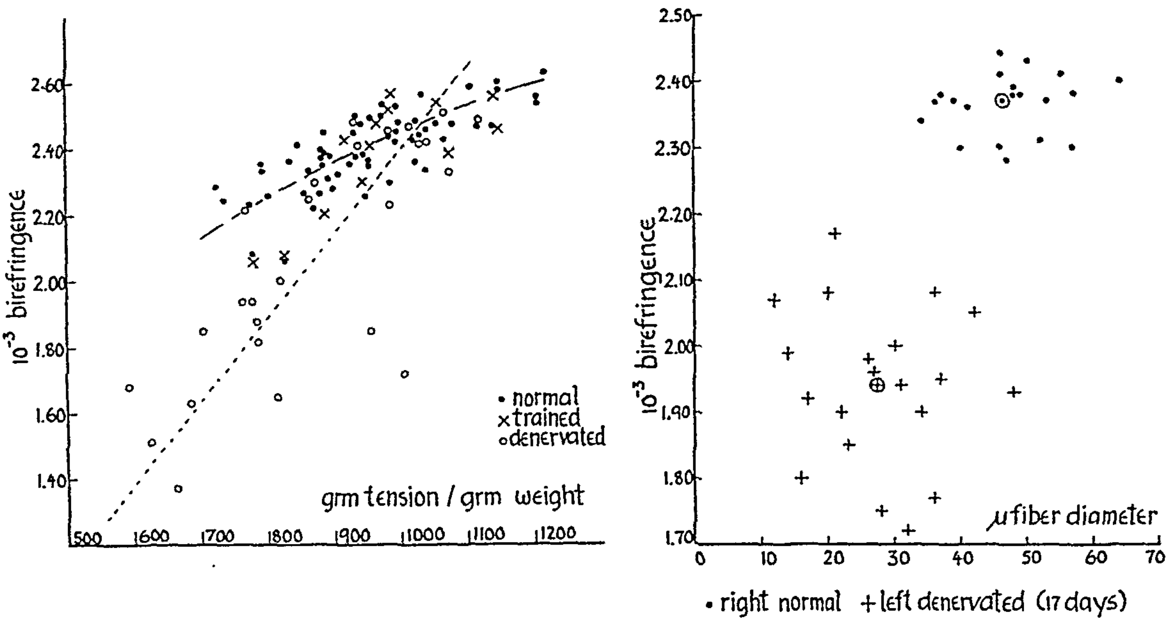

Fig. 1 Fig. 2

Fig. 1. Relation between birefringence and contractile power (gram tension per gram wet weight) for normal, hypertrophied and atrophied muscles.

Fig. 2. Birefringence values and diameters of 20 individual fibers of the normal and the atrophied partner of a gastrocnemius pair. The computed average value for each muscle is encircled.

tracile power. The measurement of the birefringence and of the diameter of various fibers of an atrophied muscle revealed without exception that there exists no parallel between diminution of fiber diameter and loss in birefringence (fig. 2). In normal muscle, as is well known, the diameters of individual fibers differ distinctly, the thickest being often twice as thick as the thinnest. The variation in birefringence is much smaller. After atrophy, the fiber diameter often varies in the proportion 1:4, and the differences in birefringence increase appreciably. However, as seen from figure 2, there is no relation between loss in birefringence and diminished fiber diameter. A rather small fiber can still have a rather high bire-

fringence while a relatively thick fiber may have a much diminished birefringence.

Discussion. Hines and Knowlton (1937) have demonstrated that in atrophying muscles the relative amount of the contractile tissue, the "muscle cell phase," and of the non-contractile tissue, the "non-muscle phase," are altered. In a recent statistical study concerning the effect of atrophy upon the strength, these authors (Knowlton and Hines, 1940) expressed the tension values per unit "muscle cell phase." From a theoretical point of view, without doubt, this method is preferable to that used in this paper. However, the determination of the "muscle cell phase" depends on an exact measurement of the water and chloride content. Knowlton and Hines, in their statistical investigation for these determinations, used muscles from other animals but at the same stage of atrophy as those rats of which the muscle strength had been measured. Such a procedure could not be used in my experiments, where individual muscles were compared. In an attempt to take into account at least to a certain extent the shift in the two phases, the strength of the muscles was related to wet weight as well as to dry weight. One must keep in mind that a strict comparison of the water content of the partners of a muscle pair is impossible under the given conditions. The vascular system of denervated or trained muscles does not respond in the same manner to muscular activity as that of normal muscles. In general, the activity hyperemia in electrically trained muscles is larger, but subsides more quickly than in normal muscles (Vanotti and Magiday, 1934) while in the denervated muscles the circulatory responses are slow and irregular.

The difference in the methods of expressing the contractile power of the muscles is only partly the reason why in my experiments contractile power does not decrease before the weight loss of the denervated muscles amounts to about 30 per cent, while Knowlton and Hines (1940) report power losses when the weight loss reaches 20 per cent. This discrepancy is due probably mainly to the fact that these authors used for stimulation electrical shocks of uniform duration for all stages of atrophy, while in my experiments the length of the single shocks was increased with proceeding atrophy, so that optimal stimulation was obtained (Fischer, 1939).

The apparent tendency to an increase in contractile power during the first stage of atrophy (table 3) is probably due to a spreading effect and an incomplete fixation of the knee joints. A more rigid fixation than that used (i.e., clamps on the exposed femurs instead of clamps on the uninjured knee joints) would have interfered too much with the restoration of normal blood supply after cessation of stimulation. During the first days of atrophy, the difference between optimally shaped electrical shocks for atrophying muscles and for normal ones is relatively high concerning threshold but still small as to duration, so that in those days an optimal

stimulation of the atrophied gastrocnemius will have a marked spreading effect upon the non-denervated muscles of the thigh. With progressing atrophy, when the duration of the shocks must be rather long and their frequency low for optimal stimulation, the thigh muscles will be much less affected by the spreading effect.

The second series of experiments reported here confirms earlier authors (for literature see Steinhaus, 1933), who report that training with faradic currents produces a muscle hypertrophy due mainly to increased fiber diameter. Why in my experiments, despite the use of a training method which should be optimal from a theoretical viewpoint, some muscles did not distinctly gain weight, and why the maximal gain observed is far under the 40 per cent reported by others, is hard to explain. It might be due to the choice of the species and muscles employed. There are no indications that I overtrained the gastrocnemii by either too strong or too long a stimulation. That such an overtraining can occur for muscles stimulated electrically has been pointed out by Lehnartz (1936). However, all trained muscles of my rats, with or without appreciable weight gain, were distinctly less fatigable than their normal partners. Since in the trained muscles neither contractile power per weight unit nor birefringence increased, the various electrical training effects upon the chemical constituents of the muscle as reported at first by Embden and Habs (1927) and studied later mainly by Russian authors (Vanotti and Magiday, 1934; Rosengart, 1936, 1937; Palladin and Raschba, 1937; Klimenko, 1937; Werbolowitsch, 1937) are probably all connected with the decrease in fatigability.

The response of the normal muscle to faradic training with its increased weight but unchanged specific strength and birefringence resembles the response of atrophied muscles to electrical treatment. As shown in an earlier paper (Fischer, 1939) such a treatment retards distinctly the weight loss of the denervated muscles but is practically unable to delay the loss in contractile power or in birefringence. The comparison of the diminution of the diameter of individual atrophying fibers with their birefringence added further evidence in support of the suggestion advanced that weight loss and destruction of the submicroscopical pattern during atrophy are two distinct processes.

The observed statistical relation between birefringence of normal muscles and their contractile power as well as the parallel diminution of birefringence and contractile power of atrophying muscle is a further indication that the submicroscopical crystalline structure of the muscle is essentially involved in the contractile mechanism. Any attempt to explain the difference between the relation of birefringence to contractile power for normal muscle and for atrophying muscle can at the moment only be based on pure speculation with little relation to other experimental data, and would be outside the scope of this Journal.

SUMMARY

1. Electrical training of rat gastrocnemii increases their weight and their total isometric strength, but does not alter their contractile power per weight unit.

2. In denervated atrophying rat gastrocnemii, the loss in total isometric strength corresponds at first to the loss in weight, thus leaving the contractile power per weight unit unaltered. This early phase of atrophy, in which also no appreciable changes in birefringence are observed, lasts 9 to 11 days, and at its end the weight losses of the muscles have reached about 30 pe cent. During the later phase of atrophy, the tension loss surpasses distinctly the weight loss. Birefringence starts to diminish too, and at about the same rate as the contractile power per weight unit. In consequence, the latter parallels the birefringence of the muscle throughout the whole course of atrophy. Even for the individual fibers, there exists no fixed relation between diminished fiber diameter and loss in birefringence.

3. For normal muscles, there exists a statistical relation between birefringence and contractile power per weight unit, which can be expressed by the equation: birefringence = k × log. contractile power.

4. The experimental data are discussed and the conclusion drawn that the submicroscopical crystalline structure of the muscle is essentially involved in the contractile mechanism.

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THE MECHANISM OF THE SECRETION OF ACID BY THE GASTRIC MUCOSA

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Hollander (1934) and Gilman and Cowgill (1931) and others have shown that the acid secretion of the gastric mucosa is pure hydrochloric acid of concentration such that it is isotonic with the blood. In forming the acid secretion the cells of the gastric mucosa of dogs raise the hydrogen ion concentration from 5×10^{-8}, the hydrogen ion concentration of blood, to 0.17 M, the hydrogen ion concentration of the acid secretion. The chloride ion concentration is raised from 0.11 M in plasma to 0.17 M in the secretion. Osmotic work is done in raising the concentration of the two ions. The minimal amount of energy needed to do the work is equal to the free energy increase obtained in raising the concentration of the ions. The free energy change can be calculated by means of the equation used by Borsook and Winegarden (1931) in their calculation of the work of the kidney. It has been found that the gastric mucosa must expend a minimum of 772 small calories per liter of secretion in concentrating the hydrogen ions and a minimum of 48 small calories per liter in concentrating the chloride ions. Since the osmotic pressures of blood and gastric juice are the same the mol fraction of water in each is the same. Consequently no reversible work is done when water passes from the blood to the secretion, and no reversible energy is expended. A very small amount of irreversible work is done on the water in overcoming viscosity when water moves at a finite rate. The energy necessary to perform this work is doubtless derived from the difference in hydrostatic pressure between the blood and the gastric juice.

Davenport (1939, 1940a, 1940b) has suggested that the mechanism of the secretion of acid is that expressed in figure 1. Some mechanism in the parietal cells whose rate of action is directly proportional to the rate of formation of carbonic acid in the cells secretes hydrogen ions and uses energy. The principle of electrical neutrality of solutions requires that exactly the same number of anions be present in the secretion as there are hydrogen ions. In order to satisfy the principle, chloride ions pass

from the plasma through the cells and into the secretion, being dragged along by the positive charge on the hydrogen ions. The chloride ions removed from the plasma are replaced by bicarbonate ions formed in the cells at the same time the hydrogen ions are formed (Bulger, Allen and Harrison, 1928). Water moves through the cells and into the secretion without osmotic work being done on it.

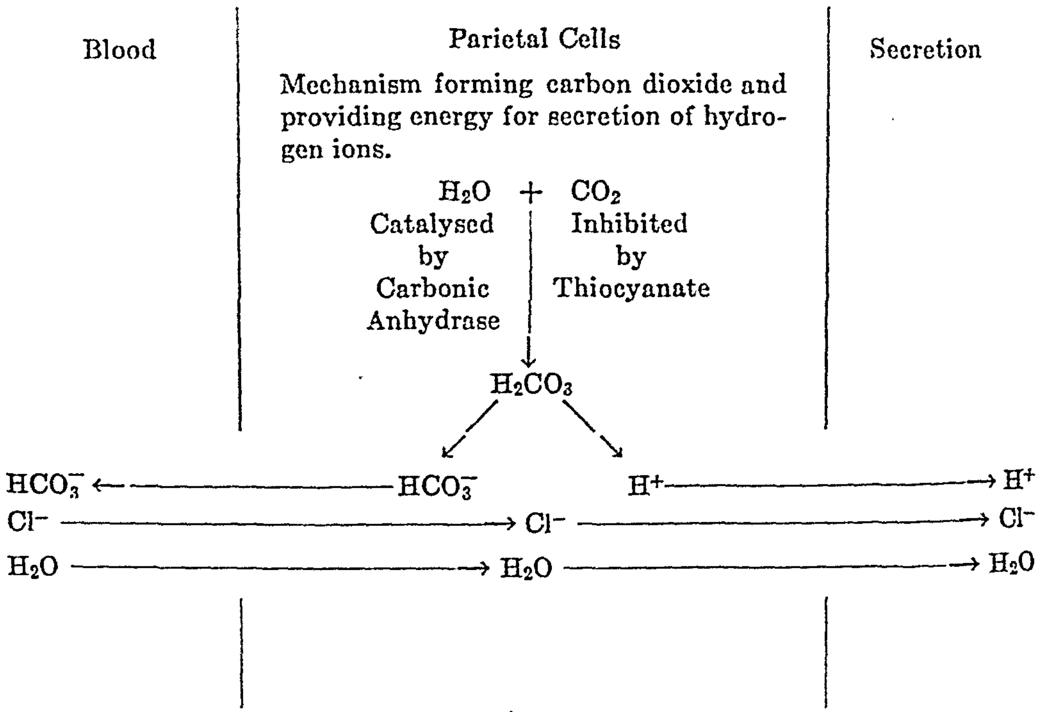

Fig. 1

The movement of chloride ions from the plasma to the secretion is in effect the transport of ions under the influence of an electrical potential difference. The velocity of the movement, u_{Cl}, is expressed by the equation

$$u_{Cl} = U'_{Cl} \frac{dE}{dl} \tag{1}$$

where dE/dl is the potential gradient and U'_{Cl} is the mobility of the ion in the cells. If it were possible to replace part of the chloride by another anion A^- the velocity of the anion, u_A, would be expressed by the equation

$$u_A = U'_A \frac{dE}{dl}. \tag{2}$$

Since the potential gradient is the same for each anion the relation between the concentrations of the anions in the plasma and gastric juice is expressed by the equation

$$\frac{[A^-]_{GJ}}{[Cl^-]_{GJ}} = \frac{[A^-]_P\, U'_A}{[Cl^-]_P\, U'_{Cl}} \tag{3}$$

where the left hand term is the ratio of the concentrations in the gastric juice and the right hand term is the ratio of the concentrations in the plasma multiplied by the ratio of the mobilities of the anions.

The mobilities of the anions in the cells and through the several membranes involved are unknown and cannot be measured. However it is reasonable to assume that they are directly proportional to the mobilities in water. This assumption is expressed by the equations $U'_A = k_A U_A$ and $U'_{Cl} = k_{Cl} U_{Cl}$ where U_A and U_{Cl} are the mobilities in water and k_A and k_{Cl} are constants. The constants express the retarding action of the cell contents and membranes upon the free movement of the anions. No doubt the magnitude of the constants depends upon the pore size of the membranes, the viscosity of the cells, the size of the anions and other factors. Since these factors can only be guessed at it is inadvisable to attempt any theoretical derivation of their magnitudes.

Substituting in equation (3) the equation

$$\frac{k_A}{k_{Cl}} = \frac{[A^-]_{GJ}\,[Cl^-]_P\; U_{Cl}}{[Cl^-]_{GJ}\,[A^-]_P\; U_A} \tag{4}$$

is obtained.

If the proposed mechanism of secretion be correct the observed ratio of k_A to k_{Cl} should be constant when the concentrations of A^- and chloride in the plasma are varied. If the anions A^- and Cl^- are retarded in passing through the cells to exactly the same extent the ratio should be equal to unity. If the anion A^- is retarded to a greater extent the ratio should be less than unity, and the degree of deviation from unity is a measure of the degree of retardation.

Equation (4) can be tested experimentally by the substitution of bromide for part of the chloride in blood. Quastel and Yates (1934) and Troenhart (1935) have shown that when bromide is present in the blood it is secreted into the gastric juice, but they made no attempt to test the mechanism of secretion in the way outlined here. Hoppe (1906) and Lipschitz (1929) found the ratio of bromide to chloride in the gastric juice to be approximately the same as that in plasma, but their data are insufficient for accurate analysis.

In the experiments reported in this paper bromide was administered to dogs. The concentrations of bromide and chloride in the gastric juice and plasma were determined. The values of the mobilities of bromide and chloride in water at various concentrations are given in Landolt-Bornstein (1923). They were plotted in the graph shown as figure 2, and the mobilities at the observed plasma concentrations were read off. The several observed quantities were substituted in equation (4), and the ratio of the constants was calculated.

It is well known that when bromide is administered it displaces chloride

from the plasma in such a fashion that the total concentration of bromide and chloride remains constant. In the animals used in these experiments $[Br^-]_P + [Cl^-]_P = 0.112$ M. Consequently for any given ratio $[Br^-]_P/[Cl^-]_P$ there are unique values of $[Br^-]_P$ and $[Cl^-]_P$, and those values can easily be calculated. By reading off the values of U_{Br} and U_{Cl} corresponding to the values of $[Br^-]_P$ and $[Cl^-]_P$ the ratio U_{Br}/U_{Cl} can be calculated for any plasma bromide to chloride ratio. By this means it was found that as the ratio $[Br^-]_P/[Cl^-]_P$ increases from 0.1 to 0.8 the ratio U_{Br}/U_{Cl} diminishes. Since it has been assumed that the actual mobilities in the secreting mechanism are directly proportional to the mobilities in water it should be found that as the ratio $[Br^-]_P/[Cl^-]_P$ increases the ratio U'_{Br}/U'_{Cl} diminishes. By substituting the observed plasma and gastric juice bromide and chloride concentrations in equation (3) the experimentally determined values of U'_{Br}/U'_{Cl} can be calculated, and the ratio should be found to diminish as the plasma bromide to chloride ratio increases. The observation of such a diminution is as important a confirmation of the theory as is the constancy of the ratio k_{Br}/k_{Cl}.

In the presentation and discussion of the results the ratio U'_{Br}/U'_{Cl} is denoted by the term *secretion ratio*, and the ratio k_{Br}/k_{Cl} is denoted by the term *secretion constant.*

METHODS. Bromide and chloride were determined by the potentiometric method of Hastings and van Dyke (1931). The method was carefully tested and found to be accurate to within 1 per cent. Thiocyanate was determined by the method of Lavietes, Bourdillon and Klinghoffer (1936). Since the solubility product of silver thiocyanate is almost identical with that of silver bromide thiocyanate is included in the titre of bromide in the potentiometric titration. When thiocyanate was present its independently determined concentration was subtracted from the apparent bromide concentration in order to obtain the true bromide concentration. The thiocyanate concentration was always small compared with that of bromide.

The gastric contents of dog 1 were sampled by means of a stomach tube. Dogs 2 and 3 were provided with Pavlov pouches. In the first group of experiments sodium bromide was added to the diets, and in the second group sodium thiocyanate was also added. At least 16 hours after feeding standard subcutaneous injections of histamine were given. During the second half-hour after injection samples of gastric juice were taken from dog 1, and samples were taken from dogs 2 and 3 as the juice was secreted. The rate of secretion by the pouch of dog 3 was measured and expressed as the number of milliliters of juice secreted during the second half-hour after injection. In that interval the rate of secretion was constant. Blood was obtained by venepuncture.

The secretion of bromide and chloride. The results obtained when bro-

mide alone was fed are presented in table 1 and in the first half of table 2 together with the calculated secretion ratios and constants. The relation of the secretion ratios and constants to the plasma bromide to chloride ratios are more clearly shown in figures 3a and 3b where they are plotted as filled circles.

The theoretical secretion ratio is equal to the ratio of the mobilities of bromide and chloride in water multiplied by the ratio k_{Br}/k_{Cl}, the secretion constant. The ratio of the mobilities in water for plasma bromide to

TABLE 1

DOG NUMBER	PLASMA			GASTRIC JUICE					SECRETION RATIO	SECRETION CONSTANT
	Cl^-	Br^-	$\frac{Br^-}{Cl^-}$	Free acid	Total acid	Cl^-	Br^-	$\frac{Br^-}{Cl^-}$	$\frac{U'_{Br}}{U'_{Cl}}$	$\frac{k_{Br}}{k_{Cl}}$
1	0.102	0.011	0.108	0.062	0.071	0.133	0.015	0.113	1.046	0.913
	0.089	0.026	0 292	0.067	0.074	0.120	0.036	0.300	1.027	0.929
	0.087	0.030	0.345	0.088	0.095	0.116	0.041	0.353	1.023	0.932
	0.082	0.029	0.354	0.083	0.092	0.118	0.043	0.364	1.028	0.936
	0.079	0.035	0.443	0.093	0.107	0.111	0.050	0.450	1.016	0.936
	0.072	0.043	0.598	0.064	0.109	0.108	0.064	0.593	0.992	0.924
	0.071	0.045	0.634	0.056	0.069	0.091	0.058	0.638	1.006	0.914
	0.068	0.046	0.677	0.053	0.064	0.090	0.060	0.667	0.985	0.925
2	0.093	0.016	0.172	0.134	0.138	0.143	0.026	0.183	1.064	0.947
	0.086	0.022	0.256	0.137	0.142	0.136	0.036	0.265	1.035	0.933
	0.091	0.026	0.286	0.113	0.128	0.131	0.038	0.290	1.014	0.916
	0.083	0.029	0.350	0.122	0.130	0.128	0.045	0.352	1.006	0.918
	0.080	0.033	0.413	0.129	0.132	0.122	0.051	0.418	1.012	0.930
	0.077	0.036	0.468	0.134	0.141	0.116	0.053	0.557	1.190	0.901
	0.074	0.040	0.540	0.122	0.132	0.110	0.060	0.545	1.009	0.936
	0.070	0.044	0.629	0.124	0.134	0.106	0.067	0.632	1.005	0.940
	0.064	0.046	0.719	0.126	0.134	0.098	0.070	0.714	0.993	0.936
	0.061	0.047	0.771	0.139	0.143	0.098	0.075	0.765	0.993	0.937
										0.928

All concentrations in mols per liter.

chloride ratios from 0.1 to 0.8 were calculated as explained above. These calculated ratios were then multiplied by the observed secretion ratio, 0.929. They were plotted as the curved line in figure 3a. The observed secretion ratios plotted as filled circles are obviously distributed at random about the theoretical line, and as the plasma bromide to chloride ratio increases the secretion ratio diminishes exactly as predicted by the theory. On the other hand the values of the secretion constant plotted as filled circles in figure 3b vary irregularly about their mean value, the deviations all lying within the narrow limits of 99.4 and 101 per cent of the mean

value of 0.929 ± 0.010. The data therefore establish without doubt that the secretion ratio diminishes as the plasma bromide to chloride ratio

TABLE 2

Uninhibited and inhibited secretion by dog 3

PLASMA				GASTRIC JUICE							SECRETION RATIO	SECRETION CONSTANT
Br^-	Cl^-	SCN^-	$\frac{Br^-}{Cl^-}$	Free acid	Total acid	Rate	Br^-	Cl^-	SCN^-	$\frac{Br^-}{Cl^-}$	$\frac{U'_{Br}}{U'_{Cl}}$	$\frac{k_{Br}}{k_{Cl}}$
0.011	0.100		0.110	0.133	0.147	14.9	0.018	0.156		0.115	1.045	0.920
0.012	0.100		0.120	0.132	0.145	15.1	0.020	0.156		0.128	1.067	0.941
0.018	0.093		0.194	0.131	0.139	15.0	0.030	0.146		0.205	1.057	0.945
0.019	0.094		0.202	0.119	0.135	14.2	0.034	0.144		0.208	1.030	0.921
0.019	0.093		0.204	0.132	0.144	15.2	0.031	0.145		0.214	1.049	0.940
0.019	0.092		0.207	0.133	0.141	14.5	0.030	0.141		0.213	1.029	0.921
0.021	0.090		0.233			14.2	0.034	0.142		0.240	1.030	0.927
0.028	0.082		0.341	0.133	0.143	15.8	0.045	0.129		0.349	1.023	0.934
0.029	0.081		0.358				0.045	0.124		0.363	1.014	0.925
0.031	0.082		0.378	0.135	0.144	14.9	0.049	0.126		0.389	1.029	0.942
0.036	0.077		0.468	0.139	0.148	16.8	0.057	0.122		0.467	0.998	0.921
0.038	0.073		0.521	0.132	0.145	16.0	0.060	0.116		0.517	0.992	0.919
0.038	0.071		0.535	0.132	0.140	15.0	0.061	0.115		0.530	0.991	0.920
0.039	0.071		0.549	0.121	0.131	15.8	0.062	0.113		0.549	1.000	0.930
0.043	0.068		0.633	0.135	0.143	15.2	0.068	0.109		0.624	0.986	0.923
0.046	0.065		0.708	0.128	0.140	16.1	0.073	0.103		0.709	1.001	0.941
0.048	0.063		0.762	0.133	0.143	15.2	0.076	0.101		0.752	0.987	0.931
				0.131	0.142	15.3 ±0.5						0.929
0.021	0.086	0.0033	0.244	0.111	0.123	12.5	0.034	0.131	0.0009	0.252	1.033	0.932
0.021	0.086	0.0044	0.244	0.103	0.121	7.8	0.033	0.129	0.0012	0.256	1.049	0.946
0.023	0.092	0.0018	0.250	0.117	0.127	12.4	0.034	0.135	0.0006	0.252	1.008	0.909
0.021	0.084	0.0042	0.250	0.109	0.119	13.1	0.034	0.131	0.0007	0.260	1.040	0.940
0.024	0.090	0.0037	0.267	0.095	0.106	9.2	0.035	0.127	0.0015	0.276	1.033	0.932
0.026	0.084	0.0042	0.310	0.093	0.113	8.2	0.038	0.121	0.0015	0.314	1.031	0.920
0.027	0.083	0.0042	0.325	0.060	0.075	5.4	0.038	0.114	0.0017	0.333	1.025	0.934
0.027	0.080	0.0036	0.338	0.117	0.127	10.6	0.040	0.118	0.0006	0.339	1.003	0.914
0.029	0.082	0.0025	0.354	0.125	0.135	14.3	0.044	0.122	0.0006	0.361	1.020	0.929
0.031	0.080	0.0011	0.388	0.125	0.133	16.4	0.047	0.119	0.0005	0.395	1.018	0.933
												0.929 ±0.011

All concentrations in mols per liter.

increases, and they demonstrate as well as can be expected that the secretion constant has a value constant within the limits of error and independent of the plasma bromide to chloride ratio. The figures further

illustrate the point that the data from all the dogs are entirely consistent, so that it may be concluded that the observed relation is a general one independent of individual variation or minor modifications in technique.

The observed constancy of the secretion constant over a wide range of concentrations clearly demonstrates that equation (4) is a correct statement of the mechanism of the secretion of bromide and chloride. Consequently it can be concluded that in all probability bromide and chloride are secreted by the same mechanism and that the mechanism acts on the ions through their negative charges in the manner postulated.

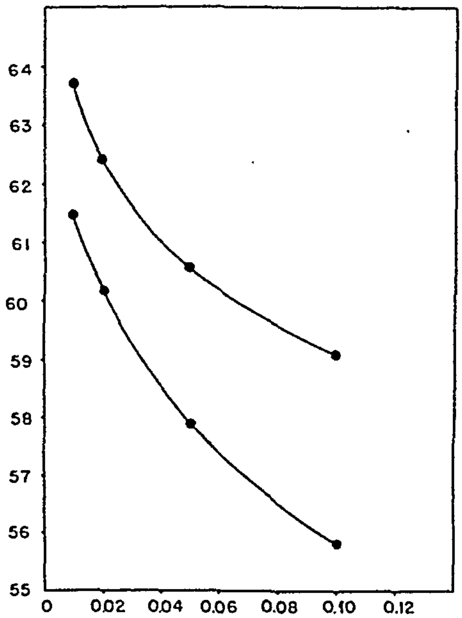

Fig. 2

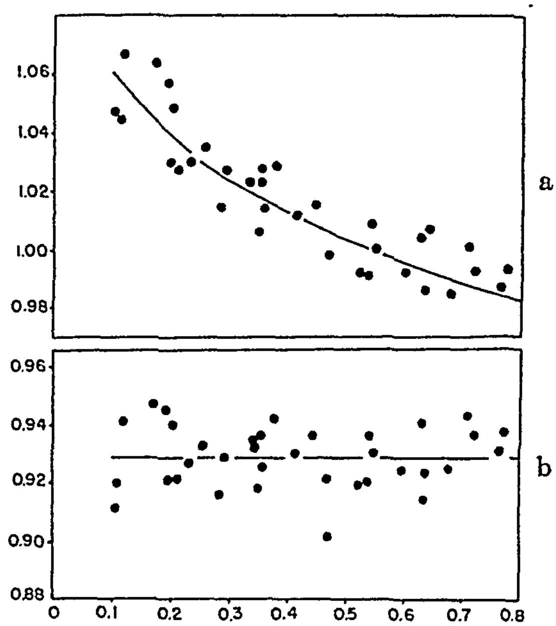

Fig. 3

Fig. 2. Ordinates: mobilities. Abscissae: molarity. Upper curve is the mobility of bromide in water; lower curve is the mobility of chloride in water.

Fig. 3a above. Ordinates: Secretion ratio. Abscissae: Ratio of bromide to chloride in the plasma.

Fig. 3b below. Ordinates: Secretion constant. Abscissae: Ratio of bromide to chloride in the plasma. Both figures are drawn to the same scale.

It may also be concluded that the assumption that the mobilities of the ions in the cells are directly proportional to their mobilities in water is correct. The secretion constant is a measure of the retardation of transport across the cells of the mucosa of bromide relative to chloride due to causes other than the differences in mobility of the ions in the cells. Since it is clear that such retardation might be expected to occur as a result of differences in the ease of passage across cell membranes it is reasonable to expect that it might bear a relation to the relative sizes of the ions. In this connection it is of interest to note that the ratio of the ionic radii of chloride and bromide derived from the data given by Pauling (1939) is

0.926 whilst the mean value of the secretion constant is 0.929. The correspondence between these values may be pure coincidence, and in any event there is no secure theoretical basis on which to found any expectation concerning the relation between ionic dimensions and transport across a cell membrane.

The data in table 1 and in the first half of table 2 also show that the acidity of the gastric secretion, the total halide concentration of the gastric secretion and the rate of secretion are entirely independent of the plasma bromide to chloride ratio. These facts strongly support the conclusion that the mechanism secreting acid is completely unaffected by the substitution of bromide for chloride. The acid secreting mechanism is indifferent to the chemical nature of the anion, and provided that an ion with a negative charge is present the mechanism is capable of secreting hydrogen ions at the same rate and at the same concentration as when chloride alone is present.

Inhibition by thiocyanate. Davenport (1940b) has shown that the carbonic anhydrase in the parietal cells and the secretion of acid are inhibited by thiocyanate ions. He postulated that the parietal cell carbonic anhydrase catalyses the hydration of carbon dioxide to carbonic acid and that the ionization of the carbonic acid provides the hydrogen ions for the acid secretion. When the rate of hydration of carbon dioxide is reduced by inhibition of the enzyme the rate of secretion of hydrogen ions is proportionately reduced.

The secretion of chloride ions is also reduced. According to the theory outlined in this paper the reason for the reduction of chloride secretion is that the diminution of the production of hydrogen ions reduces the number of positive charges in the secretion. The fewer hydrogen ions secreted the fewer anions are needed to maintain electrical neutrality of the secretion. Since the inhibition has no direct effect upon the secretion of the anions there should be no difference between the relation of bromide to chloride during normal and during inhibited secretion. Consequently it would be predicted that during inhibition the secretion ratio would bear the same relation to the plasma bromide to chloride ratio and that the secretion constant would be identical with that found during uninhibited secretion. However the total halide concentration of the secretion should be reduced. If on the other hand this theory be wrong in that the secretion of chloride is effected by some active mechanism acting directly on the chloride it would be expected that the inhibition of secretion would greatly upset the relation of bromide to chloride. Therefore the secretion ratio and secretion constant should differ from those found during uninhibited secretion.

These predictions were tested by the administration of bromide and chloride together with thiocyanate to dog 3. Bromide and thiocyanate together are more toxic than either alone, and only the lower bromide to

chloride ratios could be attained. The results are presented in table 2, and they can be compared with control observations on the same dog.

The results clearly show that when thiocyanate is present the acidity and the total halide concentration of the secreted juice and the rate of secretion are reduced. These results agree with the theory developed by Davenport (1940b) upon the postulate that as a first approximation the rate of secretion of acid is directly proportional to the rate of formation of carbonic acid in the parietal cells. However the secretion ratio varies with the plasma bromide to chloride ratio in exactly the same manner as when the secretion is not inhibited. The mean value of the secretion constant, 0.929 ± 0.011, is identical with the value 0.929 ± 0.010 obtained in the control experiments.

These results prove that the relation of bromide to chloride in the gastric juice is a function only of the relation of bromide to chloride in the plasma and of the physico-chemical properties of the anions. That relationship is not disturbed by very considerable diminution of the rate of secretion of hydrogen ions. Thiocyanate does not interfere directly with the secretion of the anions, and the total halide concentration of the gastric juice is reduced only because the secretion of hydrogen ions is reduced. These facts very strongly support the theory of acid secretion outlined in this paper, and it can be concluded that the passive character of the secretion of the anions is fully established. Further work on the mechanism of acid secretion must endeavor to explain fully the means by which the hydrogen ions are concentrated and secreted.

Conclusion. The results show that the concentration of bromide in the gastric secretion is always greater than the concentration in the plasma. In the observations on dogs 1 and 2 and in the uninhibited observations on dog 3 the ratios of bromide in the gastric juice to bromide in the plasma are on the average 1.38, 1.55 and 1.60 respectively. This means that in secreting a mol of bromide the gastric mucosa of dog 1 expended a minimum of 199 small calories, dog 2 expended a minimum of 270 small calories, and dog 3 expended a minimum of 289 small calories. Only two explanations of this fact are possible. Either the gastric mucosa contains a mechanism specifically fitted for doing work in the secretion of bromide which is called upon only when concentrations of bromide enormously in excess of the normal are experimentally produced, or the mechanism secreting chloride is capable of switching over to the secretion of bromide at any concentration with only a small loss in efficiency. The second possibility is more probably the true one. Since the chemical properties of bromide and chloride, aside from the similarity of their negative charges, are so different it is again more likely that the mechanism acting on chloride acts on the ion through the negative charge in the manner postulated and is hence also capable of acting on bromide through its negative charge.

The energy used in concentrating bromide and chloride can easily be

supplied by the mechanism secreting hydrogen ions. A minimum of 772 calories is required to secrete the hydrogen ions in a liter of acid secretion. If the hydrogen ions must pull an equal number of anions along with them the energy used in attaining the high hydrogen ion concentration is in part also used to raise the concentration of the anions. Consequently the expenditure of 820 calories on the hydrogen ions in forming one liter of secretion would result in raising the potential energy of the hydrogen ions by 772 calories and of the anions by 48 calories.

In conclusion we particularly wish to emphasize that we demonstrate in this paper the passive character of the transport of bromide and chloride from plasma to gastric juice, and that we demonstrate this passivity by showing that the two anions behave differently, the differences in behavior being explicable in terms of those physico-chemical properties of the ions which would be expected to be involved in their passive translocation. These experiments can be taken to support the view that the active process in the secretion of hydrochloric acid by the gastric mucosa is the secretion of hydrogen ions.

We are indebted to Drs. E. S. Nasset, W. B. Hawkins and S. C. Madden for preparing the Pavlov pouches.

SUMMARY

Bromide is secreted by the gastric mucosa. Over a wide range of concentrations of bromide in the plasma the rate of secretion of bromide is of the same order as the rate of secretion of chloride.

The concentration of bromide and chloride in the gastric juice is always greater than that in the plasma. Osmotic work is done in concentrating chloride, and it is concluded that the work is performed on the two anions by the same mechanism.

The rate of secretion of the gastric juice and the acidity and total halide concentration of the secretion are completely independent of the plasma bromide to chloride ratio.

The apparent mobilities of bromide and chloride in the cells of the gastric mucosa are directly proportional to their mobilities in water.

When the rate of secretion of the gastric juice and the acidity and total halide concentration are reduced by inhibition by thiocyanate the relation of bromide to chloride in the gastric juice remains exactly the same function of the plasma bromide to chloride ratio as it is during uninhibited secretion.

It is concluded that the mechanism concentrating and secreting bromide and chloride acts only through the negative charges on the ions.

An incomplete theory of the mechanism of the secretion of hydrochloric acid by the gastric mucosa is proposed.

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RESPIRATION AND GLYCOLYSIS OF RABBIT BONE MARROW IN SERUM IN RELATION TO CELLULAR COMPONENTS[1]

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A recent publication (1) has described the considerably higher and more constant rates of respiration and glycolysis of slices of rabbit bone marrow obtained when serum rather than Ringer solution is used as the suspension medium. In an earlier study (2) dealing with the oxygen consumption of rabbit bone marrow in relation to its morphology, the measurements were made in Ringer solution, and glycolysis was not studied. The present paper reports further work on the metabolism of rabbit bone marrow in relation to its cellular composition, using the improved serum technique and extending the investigation to include aerobic and anaerobic glycolysis.

The rate of respiration has been measured by the "neutralized" serum technique (1) and, where necessary, these figures have been corrected for the oxygen consumption of the serum itself, which is occasionally large enough to introduce serious errors (cf. 3 and 4). Anaerobic glycolysis in serum has been measured manometrically, allowance being made in the usual manner for the retention of CO_2 in the serum. Aerobic glycolysis has been measured in Ringer-bicarbonate-glucose solution, since the methods for doing this manometrically in serum are complicated at best (cf. 1). Even with this simplified method, one assumes that the R.Q. is unity, and as this is probably not the case, this measurement will be referred to as the "apparent aerobic glycolysis" and considered as a rough index of the aerobic glycolytic activity of the tissue without implying that it is an accurate measurement of the true aerobic glycolysis. Since the R.Q. is usually less than unity, the "apparent aerobic glycolysis" will be smaller than the true value, but useful in following relative changes.

The Q values are expressed in terms of fat-free dry weights, calculated from nitrogen determinations as previously described (1). All experiments are conducted at a temperature of 37.5°C.

Cell counts are made on smears of each marrow, prepared as described previously, except that instead of grinding the marrow slices lightly in a

[1] Supported by a grant from the Committee on Scientific Research of the American Medical Association.

mortar before smearing, the slices are now suspended in a small quantity of serum and cut finely with scissors, and Wright-Giemsa stain is now used instead of Jenner-Giemsa. At least 1500 cells are counted in each case and divided into two classes—red, or erythroid, and white, or myeloid, and the percentage of immature cells in each series is also determined. This is done by selecting a stage of development in each series, and counting cells older than this as mature and younger ones as immature. In the case of the myeloid cells the dividing line is the Class I myelocyte of Sabin and Doan (6), which is itself counted as immature, and in the red cell line, any cell which contains hemoglobin is counted as mature. These criteria of maturity have been found to be more convenient than those used in the earlier paper, but in all other respects the remarks made at that time with respect to accuracy of counts, etc., apply to the present work.

In the earlier publication, the proportion of the various cell types in the marrow was altered by selecting animals of different age, but the changes in the cell population were not as large as desirable and in the present study more drastic means have been employed to accomplish this end. These procedures will be described below, but it is to be noted that the age of the animal has been held relatively constant, in that all the rabbits used were mature—they weighed between 3 and 4 pounds and occasionally more. The rabbits were all New Zealand White males of a uniform strain; they received the usual laboratory diet of pellets supplemented occasionally with greens and were not fasted before being sacrificed for the experiments.

RESULTS. I. *The time element in the experiments.* Before proceeding with the main problems under investigation, it was considered desirable at the outset to inquire whether or not the time elapsing between the death of the animal by venesection and the beginning of the metabolic measurements was a factor influencing the results. Seven experiments were performed on different occasions in which the marrows were very rapidly sliced and placed in vessels which had already been equilibrated in the water bath. By this means it was possible to reduce the time necessary for further equilibration and the readings were started after an average interval of 48 minutes following the death of the animal. Second and third samples of the same marrows were allowed to stand in Ringer solution at room temperature, either sliced or unsliced, for further one and two-hour periods respectively before being placed in the bath, and a fourth group was allowed to stand at room temperature for an average interval of four hours after killing the animal. If the rate of respiration of the first group is considered as 100 per cent, that of the second group averaged 102 per cent, of the third group 98 per cent and of the fourth group 92 per cent. Consequently, such decrease in rate of respiration as may take place after killing the animal either occurs during the first 48 minutes (and hence is not detected by these methods) or not until between $2\frac{3}{4}$ and 4 hours at room

temperature. In all the following experiments the readings were always begun within 2½ hours after killing the animal, so that the influence of the time element, if any such exists, has been eliminated to as great an extent as possible. Similar experiments demonstrate that the same statement also applies to apparent aerobic and to anaerobic glycolysis.

Also, the morphological changes in the marrow occurring *in vivo* after death, as described by Rohr and Hafter (7), do not occur when the marrow is removed from the animal immediately after death and placed in Ringer solution. In fact, following respiration experiments in neutralized serum

TABLE 1

Respiration, glycolysis and cell counts of normal marrows

EXP. NO.	(1) Q_O	(2) $Q_G^{O_2}$	(3) $Q_G^{N_2-CO}$	(4) (3)/(1)	(5) PER CENT MYELOID CELLS	(6) PER CENT MYELOID CELLS IMMATURE	(7) PER CENT ERYTHROID CELLS IMMATURE
85	−6.3	0.6	11.5	1.82	50	10	9
86	−5.9	2.2	13.0	2.20	47	12	17
88	−6.0	2.1	13.7	2.28	53	9	10
89	−6.7	2.7	15.3	2.28	50	11	15
90	−7.6	3.1	14.7	1.94	43	10	10
91	−6.0	4.0	12.2	2.04	47	17	16
92	−5.9	2.6	11.3	1.92	40	10	10
93	−5.4	2.6	12.4	2.28	48	16	11
94	−7.2	4.3	14.3	2.00	51	13	29
95	−7.8	3.5	15.9	2.00	44	14	14
96	−6.7	4.1	16.0	2.40	66	9	10
97	−6.4	1.7	11.4	1.78	32	10	18
N-5	−5.8	2.6	13.7	2.38	56	5	10
N-6	−6.2	1.6	12.7	2.06	43	12	15
N-7	−6.7	1.9	15.5	2.31	48	8	14
N-8	−8.0	3.3	16.2	2.02	57	9	17
N-9	−6.3	2.7	15.9	2.52	56	6	14
N-10	−7.0	1.4	13.2	1.90	41	12	18
Av......	−6.6	2.7	13.8	2.09	48.5	10.7	14.3

in which the marrow has been shaken in the vessels for more than 4 hours, the cells are morphologically indistinguishable from those stained immediately after killing the animal. In the meantime, the rate of respiration has been practically constant with respect to time, as previously described (1), but in the case of glycolysis, the rate may begin to decrease after the first hour, so that only the first hour readings have been used in calculating the results given in Table 1.

II. *Normal marrows.* Table 1 shows the results obtained with 18 normal marrows. The first 3 columns indicate the rates of respiration,

apparent aerobic and anaerobic glycolysis respectively, each expressed in the conventional terms, based, however, on the fat-free dry weights calculated from nitrogen determinations. The experiments are shown individually in order that the variations between different marrows may be noted. The variation is over a fairly wide range, as is not unusual in this type of study, but it is noted that in general, in those instances in which the rate of respiration is unusually high, the rate of anaerobic glycolysis is also high, so that the ratio between these two entities (column 4) varies within a smaller range than either entity alone. Its average value is about 2.1 for normal marrows. The apparent aerobic glycolysis (column 2) shows particularly wide variations; the figures are given only to indicate that normal rabbit bone marrow does exhibit an appreciable aerobic glycolysis.

Histologically, these marrows are characterized by the myeloid and erythroid cells appearing in about equal numbers (column 5). This is a higher proportion of erythroid cells than is usually found in normal rabbits (2) (6) and is apparently a characteristic of this particular strain. The erythroid cells, however, are smaller than the myeloid cells (2), so that in these normal marrows the mass of myeloid cells is greater. There are relatively few immature cells in either series; the fact that there appear to be more immature erythroid than myeloid cells is without significance since it is due merely to the different criteria of "immaturity" chosen for the two series.

III. *Erythroid marrows.* Two groups of rabbits were treated in such a way that erythrogenesis was simulated and erythroid cells considerably outnumbered the myeloid cells in the marrow. In the first group, the animals were bled by heart puncture several times a week for various periods extending in some cases over a month. Successively large amounts of blood were withdrawn, beginning with about 20 cc. and ending with about 40 cc.; the plasma was discarded, the red cells hemolyzed with distilled water and the hemolysate reinjected into the animals intraperitoneally. These procedures induce a severe anemia and at the same time supply the materials whereby the marrow can replace the blood loss. As has been shown by Miller and Rhoads (8), the marrows come to exhibit a marked erythroid hyperplasia and the cell counts in the hemorrhagic group in table 2 indicate a corresponding decrease in the proportion of myeloid cells when compared with the normal marrows. The accompanying metabolic changes (first 4 columns) are seen to be a decrease in the rate of glycolysis, both aerobic and anaerobic, and a slight increase in the rate of respiration. The ratio between glycolysis and respiration (column 4) is therefore reduced from the normal value of 2.1 to about 1.6.

In a second group of animals a hemolytic anemia was induced by intraperitoneal injections of phenylhydrazine, usually 5 cc. of a 1 per cent solu-

tion for two doses with an intervening day, followed by an interval of several days before sacrificing the animals. Dickens has shown (9) that in addition to causing a hemolytic anemia, phenylhydrazine has a direct action on tissue metabolism characterized by an increase in aerobic glycolysis. Accordingly, the low aerobic glycolysis in the present experiments is evidence that the results are not due to a direct action of the drug on the marrow. It is also to be noted that the marrows were bright red and not brown as would have been the case if the phenylhydrazine had acted directly. The marrows showed the same morphologic and metabolic changes as in the first group but to an even greater extent, except that the rate of respiration, though averaging a little higher than normal, was not

TABLE 2

Respiration, glycolysis and cell counts of erythroid marrows

	EXP. NO.	(1) Q_{O_2}	(2) $Q_G^{O_2}$	(3) $Q_G^{N_2-CO}$	(4) (3)/(1)	(5) PER CENT MYELOID CELLS	(6) PER CENT MYELOID CELLS IMMATURE	(7) PER CENT ERYTHROID CELLS IMMATURE
Hemorrhagic group	E-6	−8.2	1.2	12.6	1.53	27	8	22
	E-7	−7.6	2.1	11.1	1.46	32	4	14
	E-8	−9.1	2.1	13.7	1.50	21	11	14
	E-9	−5.8	1.9	10.7	1.84	40	4	24
	E-10	−8.8	2.7	14.3	1.62	37	7	40
	Av.	−7.9	2.0	12.5	1.58	31	7	23
Phenyl hydrazine group	PH-2	−6.8	0.9	9.6	1.41	30	7	29
	PH-3	−6.2	1.1	7.1	1.14	18	9	43
	PH-4	−8.5	1.4	11.2	1.32	17	5	24
	PH-5	−7.2	0.7	9.0	1.25	9	9	20
	Av.	−7.2	1.0	9.2	1.28	19	8	29

quite as high as in the first group. This was more than counter-balanced, however, by the greater decrease in anaerobic glycolysis, so the ratio of the two fell to 1.3. In both groups of erythroid marrows glycolysis is evidently playing a relatively less active rôle than respiration in comparison with the state of affairs existing in normal marrows.

IV. *Myeloid marrows.* In these experiments, a wider variety of procedures was used to induce a myeloid hyperplasia in the marrows, and since in several instances more than one method was used with the same animal, there is considerable overlapping between the groups, as shown in table 3. The first group received successive large injections of saline intraperitoneally, inducing an exudation of white blood cells. Usually these were

left to degenerate or to be reabsorbed as the fluid is excreted, but in some cases the exudates were withdrawn. In any case, as is well known (10, 11), the marrow is stimulated to produce more myeloid cells. Three of these animals also received subcutaneous injections of sodium nucleinate, 25 mgm. a day, and two others received the sodium nucleinate alone, for about 20 doses. The studies of Doan and his collaborators (12, 13, 14) would lead one to suspect that this substance might have a direct effect on the marrow metabolism, but when added in high concentration directly to the marrow slices in the vessels, it did not alter their metabolism. The

TABLE 3

Respiration, glycolysis and cell counts of myeloid marrows

	EXP. NO.	(1) Q_{O_2}	(2) $Q_G^{O_2}$	(3) $Q_G^{N_2-CO}$	(4) (3)/(1)	(5) PER CENT MYELOID CELLS	(6) PER CENT MYELOID CELLS IMMATURE	(7) PER CENT ERYTHROID CELLS IMMATURE
Exudate group	M-1	−8.6	4.1	22.4	2.60	62	17	10
	M-3	−6.9	3.1	14.6	2.12	58	10	16
	M-8	−7.1	6.6	19.6	2.78	75	8	12*
	M-9	−6.7	5.0	15.0	2.24	62	11	10*
	M-6	−6.0	7.5	19.1	3.20	80	6	13
	M-10	−4.8	4.7	16.9	3.55	88	6	15
Sodium nucleinate group	M-12	−5.8	4.2	16.3	2.80	66	10	17
	M-11	−5.2	2.1	16.4	3.14	70	7	20
	M-13	−5.3	3.7	15.2	2.84	60	6	10
Infected group	M-5	−5.9	2.7	13.4	2.26	53	6	22
	M-7	−6.7	4.9	16.2	2.40	60	8	14
	A-2	−5.4	5.8	15.6	2.90	71	9	9
	A-3	−5.9	4.3	14.0	2.40	57	10	13
Average		−6.2	4.5	16.5	2.66	66	9	14

* Also had abdominal wall abscesses.

other animals were given chronic infections—2 an experimentally-induced arthritis, 2 had staphylococcus abdominal-wall abscesses, and 2 had scabies. The number of animals in each group is too small to permit conclusions as to the relative efficacy of these various procedures in inducing myeloid hyperplasia, but the best results were obtained by a combination of two methods, as indicated.

The cell counts in table 3 show that in practically all instances the myeloid hyperplasia consisted of increases in the relatively mature myeloid cells, so that the proportion of immature forms was not greater than nor-

mal. As was to be expected, the percentage of immature erythroid cells was not significantly changed. The accompanying metabolic changes are in the reverse direction from those found in the erythroid marrows—respiration tends to be slightly lower than normal and both apparent aerobic and anaerobic glycolysis are markedly increased. The average ratio between anaerobic glycolysis and respiration is raised to 2.7, indicating in these myeloid marrows a relative increase in the glycolytic activity with respect to respiration.

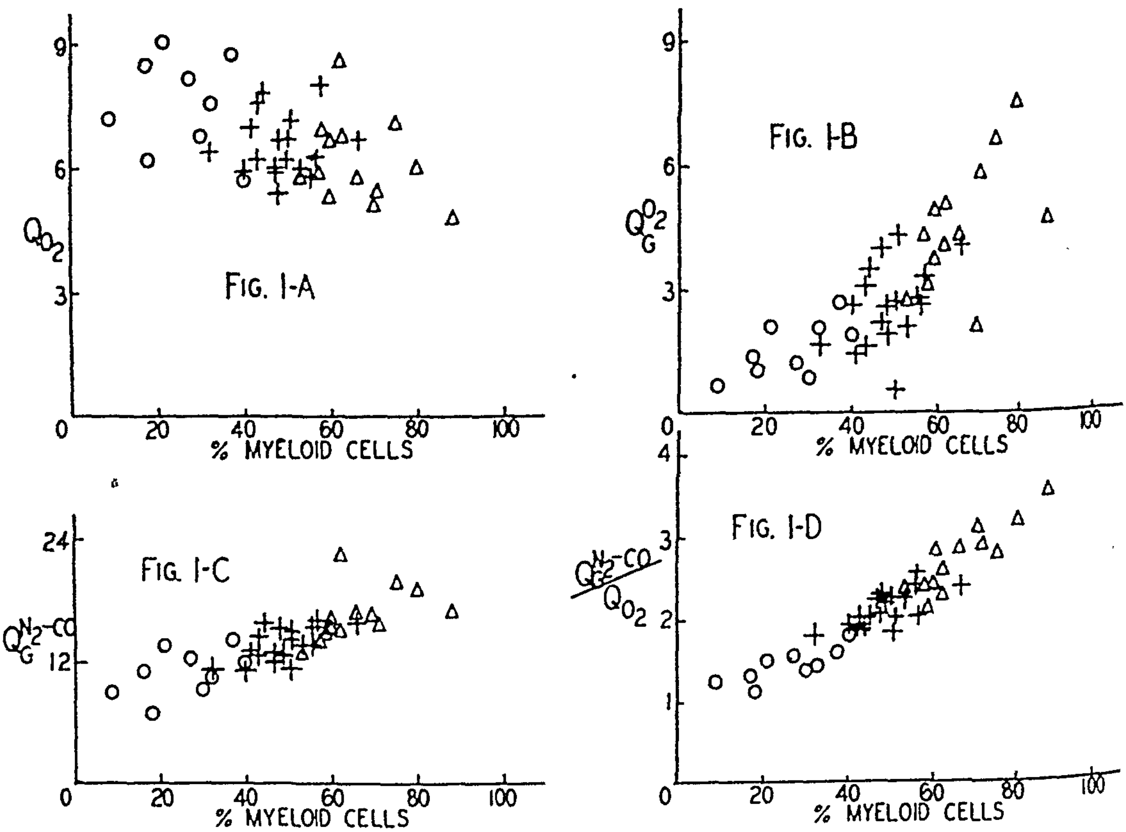

Fig. 1. Relation between respiration, glycolysis and cell counts. + Normal marrows. ○ Erythroid groups. △ Myeloid groups.

Discussion. In figure 1 the metabolic data presented in the three preceding tables are reassembled and plotted with respect to the per cent of myeloid cells. Respiration, apparent aerobic and anaerobic glycolysis are represented in figures 1–A, B and C respectively, and in figure 1–D the ratio between anaerobic glycolysis and respiration, plotted in each instance against the percentage of myeloid cells. It is clear that in the first three instances the spread of the data is very wide, so that one can speak only of trends which are followed rather than of precise relationships. Thus, in the case of respiration, there is a trend towards slightly lower values as

the percentage of myeloid cells increases, whereas in the case of aerobic and anaerobic glycolysis the trends are clearly upwards. The ratio between anaerobic glycolysis and respiration, however, shows much less variation with respect to a given percentage of myeloid cells than any one of the other individual metabolic characteristics. Accordingly, this ratio is the most useful index by which the metabolism of the marrow may be characterized—it is in the neighborhood of 2.0 for normal marrows, between 1.0 and 1.5 for markedly erythroid marrows, and in the neighborhood of 3.0 for markedly myeloid marrows. Burk (15, p. 445) has emphasized the usefulness of this particular ratio in classifying tissues generally, and tumors in particular. The relation between tumor metabolism and bone marrow metabolism will be discussed a little further on.

One may inquire whether it is possible, from these studies, to estimate the results to be expected if suspensions could be obtained consisting entirely of erythroid or myeloid marrow cells. To do this, one would have to plot lines through the points in the figures and extrapolate them to the ordinate values for 100 per cent erythroid cells and 100 per cent myeloid cells respectively. Because of the spread of the data, one obviously cannot do this with precision, but since the trends are clear, it is perhaps justifiable to make rough approximations of the general levels of the metabolism in each case. These estimates are presented in table 4. It must be pointed out that the "typical" erythroid and myeloid cells referred to in the above table would be cells of intermediate stages of maturity, not megaloblasts or myeloblasts. In fact, the marrows studied in these experiments did not contain high enough proportions of relatively immature cells of either series to enable one to establish a relation between the metabolism of the cells and their maturity.

TABLE 4

Estimates of respiration and glycolysis of erythroid and myeloid cells

	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2-CO}$
Typical erythroid cell	High moderate (approx. −9)	Very low (nearly 0)	Low (approx. 7)
Average normal marrow	Moderate (approx. −7)	Moderate (approx. 3)	Moderate (approx. 14)
Typical myeloid cell	Low moderate (approx. −6)	High (approx. 9)	High (approx. 22)

In this connection, however, it should be noted that Kempner (16) has found that the myeloblasts of human myeloblastic leukemia exhibit no

aerobic glycolysis, whereas in the present study, the normal rabbit myelocytes have a relatively high rate of aerobic glycolysis, as do leukemic myelocytes (16, 17). Whatever may be the significance of these differences, it seems desirable to emphasize that the aerobic glycolysis of rabbit bone marrow in the present experiments is not to be interpreted as due to cellular damage as considered by Fleischmann (18) and Kempner (16). It persists in serum (1), is independent of the time element in the experiments, and is merely an index, although a rough one, of the proportion of myeloid cells in the marrow. *All* the aerobic glycolysis of the marrow is probably not due to the myeloid cells, since mature erythrocytes are well known to form small amounts of lactic acid aerobically (19, 20), and some of these cells are always present in the marrow, but certainly most of the aerobic glycolysis must be due to the normal myelocytes.

It is of interest that the figures given in table 4 for the metabolism of myeloid cells closely resemble those of the same sort for tumor cells, and that indeed the ratio of anaerobic glycolysis/respiration of nearly 4.0 is also characteristic of tumor metabolism (cf. Burk (15) p. 439, column VII divided by 3). The R. Q. of these myeloid cells has not been determined, and will be important to know, but the fact that these normal myeloid cells exhibit metabolic characteristics so closely resembling those of tumor cells is merely further evidence of the impossibility of making rigid distinctions between normal and cancer cells based on such metabolic evidence alone.

In general, the moderately high rate of respiration of the erythroid cells is in line with the findings in the earlier publication (2) as well as with the studies of Orr and Stickland (5), Michelazzi (21), v. Breza (22), Schretzenmayer and Brocheler (23) and Kempner (24), but the absolute level of the Q_{O_2} in some of these studies is quite different, probably due to differences in method, species differences, and differences in the relative maturity of the particular cells being studied. In the first paper of this series (2), it was found that the rate of respiration of the marrow increased as the percentage of myeloid cells increased. However, when these rates are recalculated on the basis of marrow fat-free dry weights, this relationship is found not to apply, and it seems clear (table 4) that the myeloid cells have, if anything, slightly lower rates of respiration than the erythroid cells.

The relatively high rates of aerobic and anaerobic glycolysis of the myeloid cells is in agreement with the findings of Orr and Stickland (5) who also worked with rabbit bone marrow, and with many studies (cf. 16) on blood leucocytes. But here again, quantitative comparison of leucocyte and marrow studies is not possible at present because of the number of variables involved.

Finally, mention must be made of the significance to be attached to the

finding that the myeloid cells possess relatively active glycolytic mechanisms whereas in the erythroid cells oxidative processes predominate. The predominance of oxidative processes in the erythroid cells raises two interesting questions: 1, is the synthesis of hemoglobin necessarily an aerobic process, and 2, by what mechanism does low oxygen tension stimulate the production of cells which are relatively dependent upon oxygen for their metabolic needs?

CONCLUSIONS

1. When slices of rabbit bone marrow are suspended in serum, the ratio between the rates of anaerobic glycolysis ($Q_G^{N_2-CO}$) and respiration (Q_{O_2}) is more constant for marrows of given cellular composition than either component of the ratio alone. The relationship between this ratio and the cellular composition of the marrow has been established; the ratio is relatively high for myeloid marrows and low for erythroid marrows. Myeloid cells are therefore characterized by possessing relatively active glycolytic mechanisms, and erythroid cells by the relative predominance of oxidative over glycolytic processes.

2. Estimates have been made of the results to be expected if suspensions could be obtained consisting entirely of myeloid or erythroid marrow cells. These are, in approximate figures:

	Q_{O_2}	$Q_G^{O_2}$	$Q_G^{N_2-CO}$
For erythroid cells	−9	0	7
For myeloid cells	−6	9	22

3. The significance of these results is discussed, and it is pointed out that the aerobic glycolysis of bone marrow is not to be considered as evidence of damage to cells, or of a tumor type of metabolism, but merely as an expression of its content of myeloid cells.

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AMMONIA FORMATION IN THE AMPHIBIAN KIDNEY[1]

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The formation of ammonia by the mammalian kidney is an established function (1) and an important one in regulating the neutrality of the blood. The experiments here to be described, first determining that the amphibian kidney shared in this ability to form ammonia, were designed to determine the site of this formation. Fluid was collected from various levels of individual nephrons and analyzed. The concentrations of ammonia in glomerular fluid and fluid from all levels of the proximal tubule were too low to be measured by the analytical method employed. Only after passage through a portion of the distal tubule did significant amounts of ammonia appear in the tubule fluid, and the latter portions of this segment are therefore the site of ammonia formation. Indications were obtained that the ammonia is formed from some precursor, stored within the tubule cells.

METHODS. a. *Collection of fluid.* Frogs (*R. pipiens* and *R. catesbiana*) were found to excrete ammonia in adequate concentration under the necessary experimental conditions and were used in the majority of experiments. Necturi (*N. maculosus*) excrete ammonia in low concentrations which, during tubule fluid collection, frequently diminish to a level that made analysis impossible or uncertain. Measures calculated to produce an acidosis did not help this situation.

Fluid from the proximal segment of the frog's tubule was collected by the technique described by Richards and Walker (2). Any suitably distended segment of tubule visible on the ventral surface of the kidney was punctured and fluid collected. The relation of site of puncture to the entire tubule was subsequently determined by injecting 0.1 per cent methylene blue through the same puncture hole and observing the course which it followed along the lumen. The identification is not exact but allows decision as to the quarter of the segment punctured.

Collections from the distal tubule of the frog were more difficult; partly because of the smaller amounts of fluid within this segment, chiefly because it rarely reaches the ventral surface to become available for puncture.

[1] The expenses of this work were defrayed in large part by a grant from The Commonwealth Fund.

The occasional segments reaching the surface were visualized by two methods: 1. Methylene blue was injected into a proximal tubule and its route observed as it flowed through the distal tubule until some portion of this tubule could be seen on the kidney surface. 2. The retrograde injection of small amounts of air into the tubules from a ureteral cannula allowed visualization of any distal segments which were on the kidney surface; the position of these tubules was noted and one was punctured after the air had flowed back again into the ureter. Collections from the extreme end of the distal tubule proved impossible because its junction with the collecting duct occurred well below the kidney surface.

In the course of these experiments it became necessary to collect fluid from the collecting ducts and from the upper reaches of the ureter. To accomplish this, the dorsal surface of the kidney was exposed and brought into the microscopic field. The ureter was obstructed by a clamp placed midway between the upper and lower halves of the kidney, a pipette was inserted into it above the obstruction, and sufficient air was injected to fill the ureter and move backward into the collecting ducts. The ducts were then seen as single or branching tubes entering the ureter at irregular intervals that averaged about 1 mm. They are considerably larger than tubules and the flow of fluid down them is relatively rapid. They rarely occupied a position on the kidney surface but when such an one was observed its position was marked by neighboring chromatophores, the ureteral clamp was removed, the air allowed to reënter the ureter and the puncture performed. Collections of fluid from the ureter presented no difficulties.

Contamination of the collected specimen by fluid sucked backwards from segments of tubule distal to the site of collection was avoided by the injection of a short column of mineral oil saturated with Sharlach-R to increase its visibility. Rate of collection, determined by the rate of flow within the tubule and pressure within the collecting tip, was adjusted to keep the oil column stationary at a point just distal to the tip of the pipette. In collecting glomerular fluid we have found the use of an oil column preferable to an obstructing rod (3) but both oil and rod are necessary in experiments upon the collecting ducts because of the rapid rate of flow within these structures.

b. *Analyses of fluid.* An ultramicro modification[2] of the direct Nesslerization method has been employed which, utilizing the technique of capillary tube colorimetry described by Richards, Bordley and Walker (4), permits quantitative analysis upon 0.1 c.mm. of fluid containing as little as one-millionth of a milligram of ammonia nitrogen. The procedure involves the introduction into a glass capillary tube with inner diameter

[2] Preliminary work in the development of this method was done by Dr. Charles L. Hudson in this laboratory in 1932.

0.35 mm. of 3 separate columns of fluid of measured length separated by columns of air; a 5 mm. column of the solution containing ammonia, a 2.5 mm. column of 2 per cent gum ghatti and, last, a 2.5 mm. column of Nessler's solution without added alkali. The columns are mixed by centrifugation and the color which develops is examined by placing the capillary upon the unglazed surface of a milk glass plate in bright skylight. In the event that sufficient unknown solution is not available to provide a column of 5 mm. in length, the amounts of the reagents employed are proportionately reduced. In determining the value of an unknown solution, capillary tubes are similarly charged with ammonium chloride solutions in concentrations of 0.5, 1.0, 2.0, etc., up to 10 mgm. per cent, mixture in the unknown and standard tubes is made simultaneously by centrifugation, and the color comparison made immediately thereafter while

TABLE 1

Determinations of ammonia nitrogen in the bladder urine of frogs

CONCENTRATION OF SOLUTION		DIFFERENCE
Found	Known	
mgm. per cent NH_3-N	*mgm. per cent NH_3-N*	*per cent*
0.9	1.0	−10.0
1.8	1.7	+5.6
2.5	2.6	−3.9
3.3	2.9	+13.8
5.5	5.2	+5.8
6.3	6.0	+5.0
6.8	6.7	+1.5
7.6	7.8	−2.6
8.5	8.2	+3.5
8.3	8.4	−1.2

the color remains uniformly distributed. The lowest determinable concentration was 0.5 mgm. per cent; with concentrations exceeding 10 mgm. per cent the standards were spaced at greater intervals.

Table 1 shows the results of 10 consecutive single analyses upon frog bladder urine to which, after preliminary treatment with permutit to remove ammonia, ammonium chloride was added in amounts which were unknown to the analyst. Analyses were not made in duplicate because of the unlikelihood that tubule fluid could be collected in sufficient volume to permit duplicates. It appears that there is no systematic error and that, though the percentage error may be considerable in the lower concentrations, the absolute error need not exceed 0.4 mgm. per cent and is usually much less than this.

Several additional facts support the propriety of using direct Nessleri-

zation in capillary tube colorimetry for the determination of ammonia in amphibian urine. Frog's urine itself appears colorless when examined in capillary tubes of 0.35 mm. diameter. Its creatinine concentration, as demonstrated by Höber (5) and confirmed by analyses in this laboratory, rarely exceeds 1.0 mgm. per cent; this amount is insufficient to influence the Nessler reaction (6). Frog's bladder urine, tested with Nessler's reagent after having been shaken with permutit, is colorless; hence we conclude that possible error arising from the presence of other color-producing substances than ammonia is insignificant.

The impression was obtained that, when volumes of fluid less than 0.2 c.mm. were tested, the color which developed was less intense than that obtained with larger volumes of fluid of the same concentration; for that reason, when only small amounts were available, the standard tubes were prepared with correspondingly small amounts. The traces of protein which tubule fluid sometimes contains (4) were found to be insufficient to affect color development.

Results. The results of 31 experiments in which fluid was collected from single nephrons of 22 frogs and 2 Necturi appear in figure 1. The site of each collection is indicated by the position which the dots bear relative to the diagrammatic nephron at the top of the figure. The concentration of ammonia nitrogen in each specimen is shown as percentage of that found in specimens of urine[3] formed during the fluid collections. None of the twenty specimens collected either from glomeruli, from any level of the proximal tubule, or from the proximal quarter of the distal tubule yielded measurable intensities of color and did not therefore contain ammonia nitrogen in excess of 0.5 mgm. per cent.[4] On the contrary (table 2), five of the eight collections from the last three quarters of the distal tubule and all of three collections from the collecting ducts con-

[3] Sixteen of these urine specimens were collected from the bladder and therefore represent the combined activity of both kidneys (range of ammonia nitrogen concentration 7.0 to 18.0 mgm. per cent, average 10.6); 15 were collected from the ureter of the same kidney in which a nephron was investigated (range of concentration 3.5–14.0 mgm. per cent, average 7.5).

[4] The threshold of the method, as has been stated, is in the neighborhood of this figure. Any or all of the specimens mentioned may therefore have contained an ammonia concentration of this order and each did, in fact, develop a barely detectable trace of color which is consistent with such a conclusion. The coloration was probably due to ammonia since a series of macro analyses performed with the method of Van Slyke and Hiller (7) by Miss Ethol Shiels indicated that the arterial blood of *R. catesbiana* contained on the average 0.54 mgm. per cent. It may therefore be tentatively concluded that the low concentrations of ammonia present in frog blood are filterable through the glomerular membrane. The inability of the ultramicro method to measure these concentrations quantitatively makes this conclusion a tentative one and forces us to refer to these fluids (in table 2 and fig. 1) as being free of ammonia.

tained definite concentrations of ammonia which, in the case of the latter group, approximated those shown by ureteral urine. No experiment is included in which there was any suspicion of contamination by fluid originating distally to the site of collection or in which the approximate identity of this site was not determined.

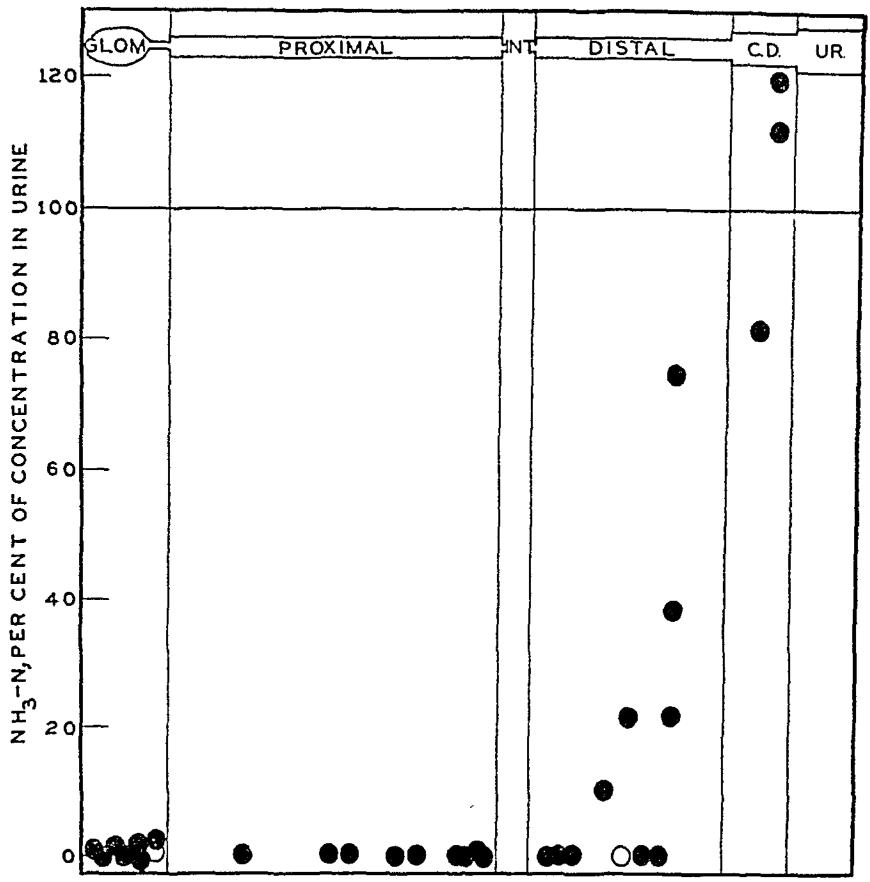

Fig. 1. Each solid dot represents an experiment upon a frog, each empty circle upon Necturus. The position of each symbol with reference to the diagrammatic nephron above indicates the portion of the unit from which the fluid was collected, its vertical position indicates the concentration of ammonia nitrogen it contained in terms of percentage of that contained by a urine specimen collected from ureter or bladder at the conclusion of the experiment. In computing these percentages a concentration of 0.5 mgm. per 100 cc. or less was regarded as 0.0 (see footnote 4). In the diagrammatic nephron: *Glom.* = glomerulus, *Int.* = intermediate tubule, *C.D.* = collecting duct, *Ur* = bladder or ureter.

These experiments prove that ammonia, other than the low concentrations which appear in blood plasma and are apparently present in glomerular fluid, does not appear within the nephron until the first quarter of the distal tubule has been passed; at about the point, that is, at which the change in pH has been shown to occur (8). Beyond this point and

throughout the remainder of the nephron there is a marked and progressive increase in ammonia concentration. This increase is far too great to be due to water reabsorption, the extent of which in the frog is accurately known (9) (10), nor do substances concentrated by this mechanism (11) show the sudden rise late in the distal tubule which ammonia presents (fig. 1). It cannot be due to secretion of ammonia from the peritubular blood supply for, in a series of 4 experiments, blood obtained from the posterior vena cava contained a higher concentration of ammonia than blood from an aortic arch[5] and (*vide infra*) ammonia continues to appear

TABLE 2

Collections of fluid from the distal tubules and collecting ducts of frogs and necturus

NUMBER	SPECIES	SITE OF COLLECTION*	RATE OF COLLECTION	AMMONIA NITROGEN IN		
				Tubule fluid	Ureteral urine	Bladder urine
			cu. mm. per hr.	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*
		Distal tubule				
1	*R. pipiens*	1/8	0.74	0.0†		18.0
2	*R. pipiens*	1/8	0.60	0.0		8.0
3	*R. pipiens*	1/4	0.51	0.0		12.0
4	*R. catesbiana*	1/3	0.20	1.4	14.0	
5	*R. pipiens*	1/2	0.19	4.0		18.0
6	*N. maculosus*	1/2	0.69	0.0	3.0	
7	*R. catesbiana*	2/3	0.24	0.0	3.5	
8	*R. catesbiana*	2/3	0.24	0.0	10.0	
9	*R. catesbiana*	3/4	0.53	2.0	9.0	
10	*R. pipiens*	3/4	0.82	1.5	4.0	
11	*R. pipiens*	3/4	0.34	3.0	4.0	
		Collecting duct				
12	*R. pipiens*	1/4	1.08	7.0	6.0	
13	*R. catesbiana*	1/2	3.00	3.5	4.0	
14	*R. pipiens*	3/4	4.50	6.0	5.0	

* The site of collection is expressed as the fraction of the segment of the nephron between its proximal end and the point of insertion of the pipette.

† 0.0 in this column means 0.5 mgm. per cent or less. See footnote 4.

in the urine when it is absent from the peritubular blood vessels. It must therefore be due to the formation of ammonia within this segment of the tubule lumen or in the cells which line it.

In an attempt to explore these latter alternatives we performed three experiments in which the kidneys of *R. catesbiana* were perfused with the fluid of Barkan, Broemser and Hahn (12); glycocoll was omitted and

[5] Average concentration of arterial blood was 0.54 and of venous blood 1.05 mgm. per cent ammonia nitrogen. The analyses were made by Miss Ethol Shiels employing the macro method of Van Slyke and Hiller (7).

the perfusion fluid therefore contained neither ammonia nor any known ammonia precursor. Ammonia continued to appear in the urine in rather high concentration (average 5 mgm. per cent) for one hour and did not completely disappear for two hours. This result is analogous to those described by Rehberg and Blem (13) during their investigation of the mechanism of urea excretion. Under these circumstances, then, the urinary ammonia must have been formed from some substance stored within the tubule cells. This substance is not ammonia itself, for we have not found appreciable amounts of ammonia in frog kidney extracts. It must therefore have been either urea or an amino acid. We were not able to distinguish between these alternatives by adding urea and glycocoll to the perfusion fluid for the excretion of ammonia, once it had ceased, could not be restored by either procedure. The existence of considerable deposits of urea within the frog kidney, demonstrated by Marshall and Crane (14), favors the suggestion that this substance is the precursor; since the deposits are said to lie chiefly within the cells of the proximal tubules and since ammonia does not appear within the tubule till well along in the distal segment, they could however only serve as the precursor if they entered the lumen as urea and were transformed into ammonia after they had progressed a considerable distance. There is no information as to the presence or absence of stores of amino acids within tubules of the frog's kidney.

One final point requires discussion: the discrepancy between the ammonia concentrations of fluid from the middle third of the distal tubule and of the urine, which is revealed by an examination of table 2, may suggest that ammonia formation also occurs in the collecting ducts, in the ureter, and even in the bladder. Our evidence opposes the latter two possibilities. Urine, allowed to stay in the bladder for two hours (with ureters ligated), did not develop any increase in ammonia concentration and, though urine from the lowest third of the ureter has frequently shown a higher ammonia concentration than that from the uppermost third, this relationship has been inconstant and appears more readily explained by differences in the degree to which various portions of a kidney concentrate ammonia under the conditions of our experiments; it is consistent with this suggestion that urine specimens, simultaneously collected from two kidneys, frequently show different ammonia concentrations. Our experiments do not exclude the collecting ducts as a possible additional site of ammonia formation but the differences in ammonia concentration between distal tubule fluid and urine would appear to be more logically explained by the three following circumstances. No fluid could be collected from the end of a distal tubule. Fluid was usually collected from tubules in which the flow was more rapid, and therefore the opportunity for ammonia concentration less, than in the majority of distal tubules. Comparison was

seldom made between fluid from a distal tubule and urine from the same portion of the kidney.

The demonstrated absence of detectable amounts of ammonia from tubule fluid until the final portions of the distal segment increases the validity of certain conclusions drawn from our former experiments with urea (15) in which the method of analysis failed to distinguish between urea and ammonia nitrogen.

SUMMARY

An ultramicro modification of the direct Nesslerization method for ammonia determinations is described which, by permitting quantitative analyses upon as little as 0.1 cu.mm. of fluid, has made possible the analysis of fluid collected from glomeruli and various portions of single renal tubules of the amphibian kidney.

Ammonia does not appear within the tubule lumen in appreciable amounts until the latter two-thirds of the distal tubule. Within this region, and possibly also in the early part of the collecting duct, the ammonia concentration gradually increases until it approximates that of ureteral urine. It is concluded that this portion of the nephron constitutes the site of ammonia formation. The source of this ammonia appears to be a precursor which is stored within the tubule cells, but the experiments did not resolve the question whether this substance is urea or an amino acid.

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VARIATION IN THE CONCENTRATION OF ACIDS OF THE GASTRIC CONTENT IN NORMAL SUBJECTS BEFORE AND FOLLOWING IMMERSION OF HAND AND ENTIRE BODY IN WATER AT VARIOUS TEMPERATURES

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In 1932 Horton and Brown, working with several patients who were hypersensitive to cold, produced, by immersing one hand of a patient in water at 10°C., a curve of gastric acidity similar to that produced by the administration of histamine. In 1937 one of us (Roth, 4) and Horton demonstrated that the rise in the concentration of gastric acidity produced by this exposure to cold approximated a subcutaneous injection of 0.4 mgm. of histamine.

A slight rise in the gastric acidity caused by the immersion of one hand in water at 10°C. has been shown in patients other than those with hypersensitiveness to cold. Therefore, the questions arose as to whether such a procedure would produce a rise of gastric acidity in normal subjects and further, whether immersion of the entire body up to the neck in water at various temperatures would produce a greater rise in the concentration of gastric acids.

Procedure. The present work was carried out on five normal subjects. Each of the five subjects was subjected to a series of tests that included immersion of one hand in water at 10°C. (50°F.) and immersion of the whole body up to the neck in water ranging in temperature, with intervals of 5° from 65 to 108°F. (18.3 to 42.2°C.). Generally, several days elapsed between the testing of each subject at the different bath temperatures to avoid the development of a refractory period. Gastric analysis accompanied these procedures in the following manner: After fasting for twelve hours samples of gastric content were obtained by means of a tube of small bore and the gastric content was evacuated at each period. A control sample was aspirated fifteen minutes after introduction of the stomach tube. With the tube remaining in the stomach, the hand of the subject or the subject was immersed in the water bath for a period of fifteen minutes. At the end of this period, before the hand of the subject or the subject was removed from the bath, another sample was aspirated. After re-

moval of the hand or the subject from the water, two more samples were obtained, one after fifteen minutes, and the other after thirty minutes. Thus, in all, four samples were obtained. Each time the subject came out of the water he was allowed to lie on a bed and was covered with the same light blanket to eliminate the effect of different room temperatures.

During all the tests the subjects carefully expectorated saliva to avoid any neutralization of gastric acidity. For the same reason, any gastric specimens which were tinged with bile were discarded. Free and total gastric acidity were determined by titration, using Töpfer's reagent and phenolpthalein as indicators and were recorded in terms of units of tenth-normal sodium hydroxide.

Results. When one hand of each of the five subjects was immersed in water at 10°C. (50°F.) for fifteen minutes and the gastric acidity was determined as previously described, only a slight rise in the gastric acidity occurred, particularly fifteen to thirty minutes after removal of the hand from the water.

When the entire body of each subject was immersed up to the neck in water ranging in temperature, with intervals of 5° on each day, from 65 to 100°F. (18.3 to 37.8°C.), the greatest rise in the gastric acidity occurred between fifteen and thirty minutes after the subject was removed from the water (fig. 1). This was particularly true when the subject was immersed in baths at temperatures from 65 to 85°F. (18.3 to 29.4°C.). At this range of bath temperature the average rise in the gastric acidity was 28.2 units of tenth-normal sodium hydroxide for total acidity and 27.5 units for free hydrochloric acid with a range of increase from 16 to 54 units of tenth-normal sodium hydroxide for total acidity and from 12 to 54 units of tenth-normal sodium hydroxide for free hydrochloric acid. Fifteen minutes after removal of the subjects from baths ranging in temperature from 90 to 108°F. (32.2 to 42.2°C.) there was only a very slight rise in the gastric acidity in three subjects and in two instances there was a decrease.

There are various reports in the literature (2, 3) that deal with the effects of baths of different temperatures on the circulation. Although most workers agree that total metabolism is increased on exposure of the body to cold baths, there is considerable controversy as to whether the increase is produced solely through muscular contractions such as shivering or by other mechanisms. When these subjects were immersed and motionless in water at 65 to 75°F. (18.3 to 23.9°C.) there was considerable shivering at first. At the other temperatures there was no visible shivering.

Although the basal metabolic rates were unchanged before immersion and at the height of the increase in the gastric acidity, fifteen minutes after the removal of a subject from a bath at 75°F. (23.9°C.), the studies were not sufficiently comprehensive to justify the drawing of conclusions from them and further metabolic studies are now being carried out.

At a temperature of 75°F. (23.9°C.) there was no visible shivering and in order to determine whether this response of the gastric acidity of normal subjects fifteen minutes after immersion in water at 75°F. (23.9°C.) was a histamine or a histamine-like reaction, histaminase was employed. Commercially[1] available histaminase is obtained from the intestinal mucosa and the kidney, and the preparation for oral use is enteric coated. Consequently, it is probable that absorption takes place only after it reaches the intestine. Therefore, a special preparation was obtained which consisted of a fine white powder that could be suspended in normal saline and could be administered by means of a duodenal tube.

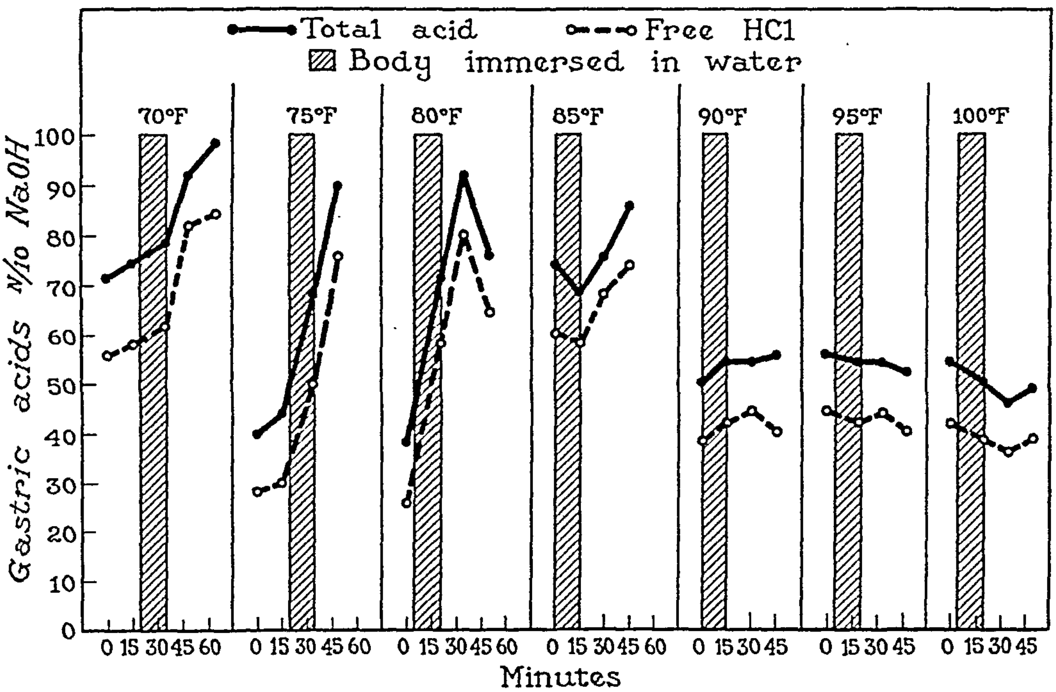

Fig. 1. The effects produced on the concentration of the gastric acidity by immersion of the body for fifteen minutes in baths at 70 to 100°F. (21.1 to 37.7°C.).

As in the previous observations, the subjects were immersed up to the neck in water at 75°F. (23.9°C.) for fifteen minutes and determinations of the gastric acidity were made before, during and for thirty minutes after the immersion in water. Several days later, histaminase was introduced into the duodenum of these same subjects twenty-five to thirty minutes before the immersion in water at the same temperature and for the same period of time. Following introduction of the histaminase into the duodenum and after subsequent washing of the histaminase into the duodenum with normal saline, the duodenal tube was withdrawn so that its tip would

[1] Histaminase was kindly supplied to us by the Medical Research Department of the Winthrop Chemical Company.

be in the stomach for aspiration of samples of gastric content. These samples were obtained at the same intervals employed in the previous procedure (fig. 2).

When 10 units of histaminase were introduced into the duodenum and samples of gastric content were obtained without immersion of the subject in water, there was little change in the concentration of gastric acidity (fig. 2*a*).

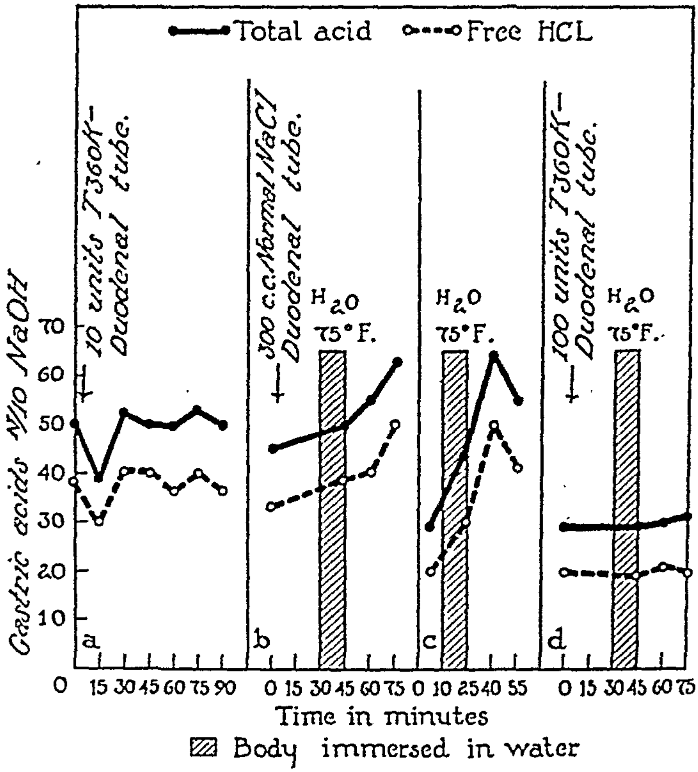

Fig. 2. Effects produced on the concentration of gastric acids; *a*, following the introduction of 10 units of histaminase into the duodenum; *b*, following the introduction of 300 cc. of physiologic saline into the duodenum thirty minutes before immersion of the subject in water at 75°F. (23.9°C.) for fifteen minutes; *c*, following immersion of the subject in a water bath at 75°F. (23.9°C.) for fifteen minutes (control observation); *d*, following the introduction of 100 units of histaminase into the duodenum thirty minutes before immersion of the subject in water at 75°F. (23.9°C.) for fifteen minutes. Note evidence of inhibitory effect of histaminase in *d*.

Since histaminase was introduced with normal saline, to rule out the factor of dilution, triple the amount of normal saline used to introduce histaminase was allowed to enter the duodenum thirty minutes before the subject was again immersed in water at 75°F. (23.9°C.). The concentration of the gastric acidity practically paralleled that present at the time of the control immersion of the subject (fig. 2*b* and *c*).

In each of these five subjects, when 100 units of histaminase were in-

troduced into the duodenum by means of the duodenal tube thirty minutes before the subject was immersed in water at 75°F. (23.9°C.), the usual increase in the concentration of gastric acidity following immersion of the subject in water at this temperature did not occur. Since this rise in the concentration of gastric acidity was inhibited by the introduction of histaminase, it would seem logical to conclude that the previous rise in gastric acidity most likely was produced by histamine or a histamine-like substance (fig. 2*d*).

SUMMARY

When the hand of each of five normal subjects was immersed in water at 10°C., there was only a slight increase in the gastric acidity during and fifteen minutes after the hand was removed from the water.

When these five normal subjects were immersed up to the neck in water ranging in temperature, with intervals of 5° on each day, from 65 to 85°F. (18.3 to 29.4°C.), a definite rise in the gastric acidity occurred particularly fifteen minutes after removal of the subject from the water.

Fifteen minutes after removal of the five normal subjects from water ranging in temperature, with intervals of 5° on each day from 90 to 108°F. (32.2 to 42.2°C.), only a very slight rise in the concentration of gastric acidity occurred in three subjects while there was a definite decrease in the gastric acidity in the other two subjects.

When histaminase was introduced into the duodenum by means of a duodenal tube thirty minutes before immersion of each of the five subjects in water at 75°F. (23.9°C.), the usual rise in the gastric acidity produced by water at this temperature was inhibited. This would seem to indicate that the rise in gastric acidity was probably due to the release of histamine or a histamine-like substance.

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SPECIES DIFFERENCES IN THE CHOLINERGIC ACTION OF ESTROGEN[1]

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Injection of estrogen into ovariectomized rabbits is followed within an hour by an increase in the free acetylcholine content of the uterus (Reynolds, 1939a; Reynolds and Foster, 1939). This is, accordingly, an example of the cholinergic action of estrogen in this species, and one which is demonstrable in the transplanted uterus, free of connection with the central nervous system (Reynolds, 1939a). In rabbits, there is also evidence that the sympathetic nerves to the uterus are cholinergic (Sherif, 1935; Reynolds, 1939b). Since, however, the sympathetic innervation of the uterus of the cat is adrenergic (Cannon and Bacq, 1931; Cannon and Rosenblueth, 1936), it was considered desirable to ascertain whether or not estrogen exerts a similar cholinergic action on the uterus of the cat. The following experiments record the results of such observations. In addition, experiments have been carried out on the uterus of the rat since there is evidence which purports to show by indirect means that in this species estrogen may not act in association with acetylcholine in the uterus (Holden, 1939; Astwood, 1940).

PROCEDURES. Ovariectomized animals were used. The operation was carried out from six to seventy days before the experiment, although eighteen to twenty-one days was most often the elapsed time between operation and experiment. In all, twenty-six cats and fifty-two rats were used in this work. Eleven of the cats were untreated controls, while fifteen were treated with estrogen. Twenty-one of the rats were used as untreated controls, thirty-one were treated with estrogen. Inasmuch as the rat uteri were very small, it was necessary to group them into lots of two to four uteri each. Seven of these were extracts from control rats, ten were from estrogen-injected ones.

The most frequently used estrogen was Amniotin (Squibb), as in the earlier experiments on rabbits, although Progynon-B (Schering) or Ben-Ovocylin (Ciba) was the hormone employed in a few experiments. There is no demonstrable difference between these preparations in their cholinergic action on the uterus of the rabbit (Reynolds and Foster, 1939).

[1] Aided by grants from the committee for Research in Problems of Sex, National Research Council, and the Josiah Macy, Jr. Foundation.

The amounts of hormone used were for the most part 10,000 rat units of Amniotin and 10,000 international units of the estradiol-benzoate products. Smaller quantities were employed in the early experiments until it was apparent that they were without effect upon the acetylcholine-content of the uterus. In the rabbit, it should be noted, 100 rat units of Amniotin per kilogram of bodyweight regularly increased the concentration of free acetylcholine in the uterus.

The method of extraction of the uterine tissues and of testing the extracts for an acetylcholine-like substance was exactly as in the earlier experiments (Reynolds, 1939a; Reynolds and Foster, 1939). It is the technic recommended by Chang and Gaddum (1933). The sensitivity of the frog's rectus abdominis muscle (the test-object employed) varied from 0.01 to 0.05 gamma of acetylcholine. Two experiments, not included in

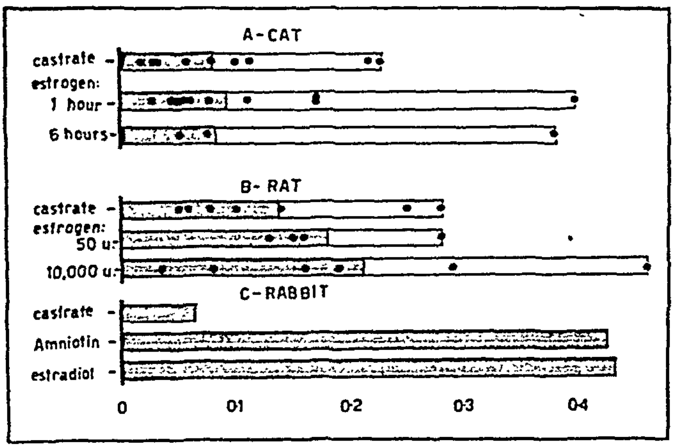

Fig. 1. Chart showing the absence of a cholinergic effect on the uterus of the cat and rat, contrasted with that seen in the rabbit. Dots, tests of individual extracts. Shaded areas, mean concentrations of an acetylcholine-like substance. Figures, gammas per gram of fresh tissue.

the groups above, were discarded because the least discernible response of the test-muscle was to 1.0 gamma of acetylcholine. The concentration of the extracts was one cubic centimeter for 0.3 to 1.5 grams of fresh tissue.

Results. *In cats.* It will be seen in figure 1A that no difference was found in the acetylcholine-equivalent content of uteri from untreated and from estrogen-treated cats. This is indicated by the mean values obtained, and by the range of the individual points in each group of experiments. The mean concentration was found to be 0.08 to 0.09 gamma of an acetylcholine-like substance per gram of fresh tissue. In the untreated rabbit uterus, the concentration of such a substance is about the same (0.06–0.07 gamma per gram of fresh tissue). In contrast, the mean concentrations of acetylcholine in the uteri of estrogen-injected rabbits ranged from 0.42 to 0.79 gamma per gram of fresh tissue in different groups of experiments (Reynolds and Foster, 1939).

In order to exclude the likelihood that the time-course of a cholinergic effect in the cat differed appreciably from that found in the rabbit (where the maximum concentration of acetylcholine is observed one hour after injection of the hormone), five experiments were carried out with uteri taken six hours after injection of the estrogen. As will be seen in figure 1A, no increase in the amount of an active substance was found. One must therefore conclude that estrogen administered to cats, unlike the situation in rabbits, is without a cholinergic action upon the uterus.

In rats. In figure 1B, it will be seen that there is no apparent difference between the quantities of an acetylcholine-like substance in rat uteri before and after estrogen. The concentration of such a substance is about double that observed in uteri from castrated cats and from untreated, ovariectomized rabbits.

No attempt has been made in this work to correlate the values obtained with anything but the wet-weight of the organ. This was found to be adequate in the case of the rabbit. If the concentration of an acetylcholine-like substance in the uterus were correlated with the dry-weight, or perhaps with the nitrogen, or some other tissue constituent, significant differences in this respect possibly might be demonstrable. In view of the data available, however, we must conclude that a species difference clearly exists with regard to the cholinergic action of estrogen in the rabbit, on the one hand, and in the cat and rat, on the other.

SUMMARY

1. Estrogen has a cholinergic action on the uterus of the rabbit, independently of connections with the central nervous system.
2. In cats and rats, it is found that estrogen is devoid of a cholinergic action on the uterus.
3. Attention is called to the fact that in rabbits, both estrogen and the sympathetic nerves to the uterus are cholinergic, whereas in the cat, estrogen is without a cholinergic action on the uterus, and the sympathetic nerves to the uterus are adrenergic. The character of the uterine innervation in the rat has not been established.

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NATURAL ISOHEMAGGLUTINATION IN DOGS*

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The phenomenon of isohemagglutination as described by Landsteiner (1) in 1900 in human beings was not as readily demonstrated in dogs. Von Dungern and Hirschfeld in 1910 (2) reported that distinct isoagglutination does not occur in the normal dog. They, however, demonstrated differences between erythrocytes of different dogs by the use of human typing serum, and thereby attempted to establish specific blood groups in dogs. The first to show that natural isohemagglutination occurred in dogs was Ottenberg, Friedman and Kaliski (3) in 1911, and they called it a weak phenomenon. Later studies by them (4) showed the dangers of incompatible transfusions between dogs. The most extensive work on natural isohemagglutination in dogs was done by McEnery, Ivy and Pechous (5) in 1924. They reported positive agglutinations in 533 instances out of 2,270 tests, but an attempt to establish groups was unsuccessful. On the other hand, Lauer (6) in 1930 obtained no positive agglutinations in over 200 tests. Recently Melnick, Burack and Cowgill (7) reported development of isohemagglutination in dogs following repeated injections of red blood cells of other dogs, upon which Melnick and Cowgill (8) in 1937 established the occurrence in dogs of two blood groups. The production of isohemagglutinins was confirmed by Wright in 1936 (9).[1]

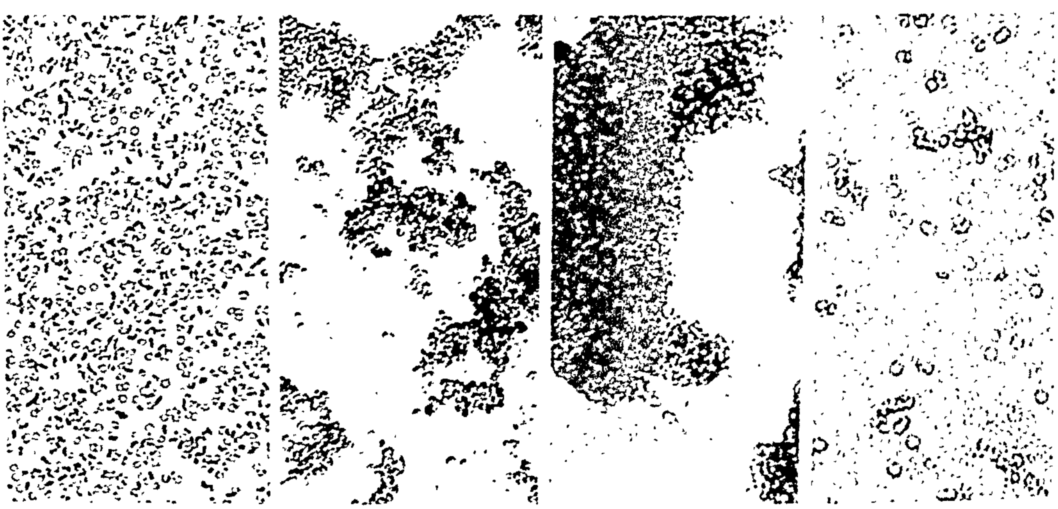
Hemagglutination is a simple and clear cut phenomenon, and we were impressed by the fact that so much conflict of opinion existed concerning its occurrence in the dog. In numerous experiments on dogs in which blood, serum and plasma transfusions were employed (10) we discovered the essential factor in hemagglutinations in dogs: namely, that the isohemagglutinins are present in plasma but absent in the serum of dogs' blood. All the above investigators either stated or implied that blood serum was used in doing their agglutination tests.

* Aided by the Kuppenheimer Fund.

A preliminary report was read at the meeting of the American Physiological Society in New Orleans, March, 1940.

[1] A paper by B. Zvetkov "The Blood Groups of the Dog" Bjuleteni postijnoi Komisii Viccannja Krovjanich ugrupovan 1927, 1, 59-65, could not be obtained.

Methods. Normal healthy dogs were picked at random; consequently a variety of animals as to breed, sex and age was observed. In each experiment, groups of nine to twelve animals were tested at one time against each other, i.e., 81 to 144 compatibility tests with plasma and double this number when parallel tests were made with serum. Care was exercised in obtaining blood for plasma by having the anticoagulating agent in the syringe. Various anticoagulating agents were employed and all seemed to be equally satisfactory. In most of this work sodium citrate has been employed. The erythrocytes rarely hemolyzed when placed in a mixture of sodium citrate and physiological saline solution. The Vincent open



A B C D

Fig. 1. (Micro-photographs—App. 1:100 Mag.) Typical reactions of dog erythrocytes when mixed with plasma using the Vincent open slide method.

Reading from left to right, A, B, C, D.

A. Negative reaction (−). No agglutination.

B. Positive reaction (+). Definite agglutination.

C. Strong positive reaction (++). Heavy agglutination.

D. Questionable reaction (∓). Little if any agglutination. Chiefly Rouleaux formation.

slide method was used in most of our work. Attempts had been made to employ Landsteiner's centrifuge method and when successful it checked the Vincent method. The difficulty in utilizing Landsteiner's procedure is the great tendency of dogs' erythrocytes to hemolyze. This hemolysis did not seem to have any relation to the phenomenon of isoagglutination. All tests were checked microscopically and were evaluated as follows: no agglutination, negative (−) (fig. 1 A), definite agglutination, one plus (+) (fig. 1 B) heavy agglutination, two plus (++) (fig. 1 C). Some agglutinations were doubtful (±) (fig. 1 D), and were counted as negative (v.i.).

Results. Ninety-seven dogs were used and a total of 3355 compatibility tests were done. Breed, sex and age of the animals seemed to have no influence on the occurrence of natural isohemagglutination. In 2281 tests blood plasma was used as agglutinating fluid, with 974 or 42 per cent positive agglutinations. In 1074 tests blood serum was used as agglutinating fluid, with only 63 or 6 per cent positive agglutinations. Parallel tests on the plasmas corresponding to the serums resulted in 462 positive results, or 43 per cent. Table 1 illustrates a typical experiment on 12 dogs, using plasma as agglutinating fluid. Plasma and erythrocytes of each animal were tested against plasma and erythrocytes of all other animals in this group. The horizontal columns present the effects on the red cells of each dog of the plasma of all other dogs of the group. The vertical columns present the effects of the plasma of the individual dog

TABLE 1

Iso-agglutination in the plasma of a group of dogs

CELLS OF DOG NO.	PLASMA OF DOG NO.											
	1	2	3	4	5	6	7	8	9	10	11	12
1	−	++	−	++	±	++	−	+	++	+	+	+
2	−	−	−	−	−	−	−	−	±	±	−	−
3	−	++	−	+	−	+	++	++	+	±	++	−
4	−	±	−	−	−	±	±	−	−	+	−	−
5	−	++	−	++	−	++	+	+	+	+	+	−
6	−	±	−	±	−	−	−	−	−	+	−	+
7	−	+	+	+	−	++	−	+	+	++	−	−
8	−	−	−	−	−	±	+	−	−	−	−	−
9	−	+	−	+	−	+	+	−	−	++	−	±
10	−	++	±	++	−	±	+	+	+	−	+	±
11	−	+	−	+	±	+	−	+	−	+	−	+
12	−	++	−	++	−	++	+	+	+	+	+	−

TABLE 2

Iso-agglutination in the serum of a group of dogs

CELLS OF DOG NO.	SERUM OF DOG NO.											
	1	2	3	4	5	6	7	8	9	10	11	12
1	−	−	−	±	−	±	−	−	−	−	−	−
2	−	−	−	−	−	−	−	−	−	−	−	−
3	−	−	−	−	−	−	−	−	−	−	−	−
4	−	−	−	−	−	−	−	−	−	−	−	−
5	−	−	−	+	−	±	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−	−	−	−
7	−	−	−	−	−	−	−	−	−	−	−	−
8	−	−	−	−	−	−	−	−	−	−	−	−
9	−	−	−	−	−	−	−	−	−	−	−	−
10	−	−	−	±	−	−	−	−	−	−	−	−
11	−	−	−	−	−	−	−	−	−	−	−	−
12	−	−	−	+	−	−	−	−	−	−	−	−

on the erythrocytes of all other dogs of the group. Fifty-nine definite agglutinations were observed, an incidence of 41 per cent. Table 2 illustrates a typical experiment using serum as the agglutinating fluid performed on the same dogs and at the same time as the plasma agglutinations in table 1. Only two definite agglutinations were observed, an incidence of 1.4 per cent.

Repeated tests were done on the same dog using plasma as the agglutinating fluid in order to learn whether the phenomenon of isoagglutination was constant or only transient in the individual dog. Only questionable changes occurred in many repeated tests on the same animals, and these appeared to be associated with the health of the animal. Canine distemper and its complications definitely affected isoagglutination. On several occasions distemper caused pseudo- or auto-agglutination, between erythro-

cytes and plasma or serum obtained from the same dog. This latter phenomenon was not observed in healthy animals. Seven healthy dogs were observed over a six months' period and identical results were obtained in frequent compatibility tests.

The incidence of positive agglutinations with blood serum was much higher in the early stages of this study. This was due to the fact that at that time the serum was improperly prepared, i.e., the fibrinogen was not completely removed. When freshly clotted blood was centrifuged at high speed, the resultant serum was not completely free of fibrinogen, and resulted in 45 positive agglutinations in 308 tests, an incidence of 15 per cent. This incidence was reduced markedly by allowing the blood to stand for 12 to 16 hours during which time more fibrin precipitated out; at this time only 10 agglutinations occurred in 624 tests, an incidence of 1.6 per cent as compared to the 15 per cent agglutinations from serum which still contained fibrinogen. In a further effort to completely remove all fibrinogen, serum obtained from recalcified citrated plasma was used, with not a single instance of agglutination in 243 consecutive tests. This confirms Lauer's work (6) previously mentioned. McEnery's (5) 23 per cent incidence of agglutination was probably obtained with serum containing fibrinogen. This is supported by McEnery's remark that on standing for 1 week, the serum lost its agglutinating power. This loss of agglutinating power is obviously due to the fact, frequently observed in our studies, that on standing fibrin will precipitate out from serum prepared quickly from freshly clotted blood. Even citrated plasma, on standing, will lose its fibrinogen and, with it, the property of agglutinating red blood cells. Table 3 illustrates a typical experiment showing the rôle of fibrinogen in iso-agglutination.

The agglutinated erythrocytes appear to be more fragile than those remaining free. When partial hemolysis[2] has occurred, the remaining intact red blood cells show a greatly diminished tendency to agglutinate. In the experiment presented in table 4, 38 positive and 5 questionable results in 81 tests on non-hemolyzed blood were reduced to 3 positive and 11 questionable results, by hemolyzing 75 to 80 per cent of the cells; positive agglutination of such cells appeared only on standing for 15 minutes. Therefore partially hemolyzed blood should not be used for agglutination tests if accurate results are desired. Great care must be exercised when working with dogs' blood for their erythrocytes are more fragile than those of man. This readiness to hemolyze has been observed by other investigators (3).

In analyzing our work, we noted that the incidence of isohemagglutination was similar in certain animals thus indicating the possibility that blood groups may exist in dogs. In order to classify different animals as

[2] Hypotonic saline solution was used.

belonging to one group, three conditions should be met. Their blood should be mutually compatible; their erythrocytes should react similarly

TABLE 3

Rôle of fibrinogen in iso-agglutination. A. Citrated plasma

CELLS OF DOG NO.	PLASMA OF DOG NO.								
	13	14	15	16	6	17	18	19	20
13	−	−	+	−	+	+	−	+	++
14	+	−	±	−	+	+	−	+	++
15	+	−	−	−	+	+	−	+	+
16	+	−	−	−	+	+	+	+	+
6	+	+	−	−	−	−	−	+	−
17	±	−	+	−	−	−	−	−	−
18	+	−	±	−	+	+	−	+	±
19	±	−	+	−	±	+	−	−	+
20	±	−	−	−	−	−	−	−	−

Rôle of fibrinogen in iso-agglutination. B. Serum from clotted blood

CELLS OF DOG NO.	SERUM OF DOG NO.								
	13	14	15	16	6	17	18	19	20
13	−	−	±	−	±	−	−	−	+
14	−	−	−	−	−	−	−	±	+
15	+	−	−	−	−	−	−	−	±
16	−	−	−	−	−	+	−	±	−
6	−	−	−	−	−	−	−	−	−
17	−	−	−	−	−	−	−	−	−
18	−	−	−	−	+	−	−	−	−
19	−	−	−	−	−	−	−	−	−
20	−	−	−	−	−	−	−	−	−

Rôle of fibrinogen in iso-agglutination. C. Serum from recalcified plasma

CELLS OF DOG NO.	SERUM OF DOG NO.								
	13	14	15	16	6	17	18	19	20
13	−	−	−	−	−	−	−	−	−
14	−	−	−	−	−	−	−	−	−
15	−	−	−	−	−	−	−	−	−
16	−	−	−	−	−	−	−	−	−
6	−	−	−	−	−	−	−	−	−
17	−	−	−	−	−	−	−	−	−
18	−	−	−	−	−	−	−	−	−
19	−	−	−	−	−	−	−	−	−
	−	−	−	−	−	−	−	−	−

TABLE 4

Controls for iso-agglutination in non-hemolyzed blood

CELLS OF DOG NO.	PLASMA OF DOG NO.								
	13	14	15	16	6	17	18	19	20
13	−	−	+	−	+	+	−	+	++
14	±	−	±	±	+	+	−	+	++
15	+	−	−	−	+	+	−	+	+
16	+	+	+	−	++	+	+	+	++
6	+	−	+	−	−	−	+	+	−
17	+	−	−	−	−	−	−	+	−
18	+	−	±	−	+	+	−	±	+
19	+	−	+	−	+	+	+	−	+
20	+	−	−	−	−	−	−	−	−

Iso-agglutination in partly hemolyzed blood (75 to 80% hemolysis)

CELLS OF DOG NO.	PLASMA OF DOG NO.								
	13	14	15	16	6	17	18	19	20
13	−	−	±	−	±	−	−	−	±
14	−	−	−	−	−	−	−	−	+
15	−	−	−	−	−	−	−	−	±
16	+	−	±	−	±	−	±	±	+
6	−	−	−	−	−	−	−	−	−
17	−	−	−	−	−	−	−	−	−
18	−	−	−	−	−	−	−	−	−
19	±	−	−	−	−	−	−	−	±
20	±	−	−	−	−	−	−	−	−

with other plasmas, and their plasma or serum should react alike with other red cells. Table 1 shows that the bloods of dogs 1, 3 and 5 reacted

in identical fashion. In table 5 the results from table 1 have been regrouped. The blood of each dog in group I fulfills the three criteria mentioned above, i.e., they may belong to one blood group. This group is comparable to the AB type in humans using Landsteiner's nomenclature, because the plasma agglutinates no other cells and the cells are agglutinated by all plasmas of other possible groups. The blood from dogs 2, 4, 6 and 8 reacted entirely different from those in group I. They are compatible with each other and their red cells and plasma react similarly. This group is comparable to the O type in the human. The remaining dogs seem to belong to an intermediate group consisting of 2 or more groups, which may be separated in the future.

TABLE 5

Possible grouping of dogs

GROUP NO.	CELLS OF DOG NO.	PLASMA OF DOG NO.											
		Group I			Group II				Intermediate group				
		1	3	5	2	4	6	8	7	9	10	11	12
I	1	−	−	±	++	++	++	+	−	++	+	+	+
	3	−	−	−	++	+	+	++	+	+	±	++	−
	5	−	−	−	++	++	++	+	+	+	+	+	−
II	2	−	−	−	−	−	−	−	−	±	±	−	−
	4	−	−	−	±	−	−	−	−	−	+	−	−
	6	−	−	−	+	−	−	−	−	−	+	−	+
	8	−	−	−	−	−	−	−	+	−	−	−	−
Intermediate	7	−	+	−	+	+	++	+	−	+	++	−	−
	9	−	−	−	+	+	+	−	+	−	+	−	±
	10	−	−	−	++	++	±	+	+	+	−	+	±
	11	−	−	−	+	+	+	+	−	−	+	−	+
	12	−	−	−	++	++	++	+	+	+	±	+	−

The effect of transfusion of blood whose incompatibility had been demonstrated in vitro by iso-agglutination with plasma, was observed in several experiments. Different types of reactions were noted. In the most severe type the animal went into a profound shock before the infusion was completed and died following convulsions and paralysis four hours after the transfusion. Another type of reaction was less severe, and would have passed unnoticed if blood pressure had not been taken throughout and following the period of infusion. It consisted of a transient drop in blood pressure of 50 to 80 mm. Hg from which the animal recovered within one hour after transfusion. In other cases although there was no effect on blood pressure other reactions were noted, such as anuria and hemoglobinuria which sometimes cleared up after several days. There is no

doubt, however, that in many cases transfusion of incompatible blood will produce no symptoms in dogs. This is due to the weakness of the natural isohemagglutination in dogs. The titer of the agglutinins in dog's blood is approximately 1:4, whereas in man the titer is much higher.

SUMMARY

1. The blood plasma of dogs contains natural isohemagglutinins which are absent in the blood serum.
2. Complete removal of fibrinogen from the plasma removes the property of hemagglutination.
3. Those erythrocytes in the dog that are agglutinable are the most fragile cells.
4. It is believed that dogs may be divided into blood groups.

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THE MOBILIZATION OF VITAMIN A BY THE SYMPATHICO-ADRENAL SYSTEM[1]

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Drummond and MacWalter reported in 1934 that the removal of a single lobe of liver in the rat results in a large decrease in the vitamin A and carotene content of the remainder of the organ. These workers had attempted to follow the conversion of injected carotene to vitamin A *in vivo*, using the extirpated lobe as a control. The changes induced by the operation itself balked this procedure.

In the course of a study of carotene metabolism in rabbits we have performed similar experiments. In our initial work a lobe of liver was tied off and removed under ethyl urethane anesthesia. Its carotene and vitamin A concentrations were determined immediately by methods described below. Three to five hours following this operation the animal was killed with a blow on the head, and the remainder of the liver was quickly removed and similarly analyzed. The vitamin A and carotene content of the second portion of liver had invariably decreased, the vitamin A by an average of 23 per cent, the carotene by about 50 per cent (table 1).

There is no obvious reason why any part of this procedure should have promoted the actual destruction of carotene and vitamin A. It is more probable that the rapid removal of these substances from the liver is due to their transport to other tissues. This consideration led us to repeat the experiments, adding simultaneous determinations of blood vitamin A. Blood carotene is so low in these animals that we were unable to estimate it reliably.

The animals first were fasted for 24 hours in order to eliminate variations in blood vitamin A due to absorption from the intestine. Just before the removal of a lobe of liver, 10 ml. of blood were drawn from an ear vein under anesthesia. Another 10 ml. of blood were taken from the same animal just before it was killed to obtain the remaining liver tissue. The vitamin A content of each sample was determined at once (v. below). Simultaneous with its fall in the liver, the vitamin A concentration of the blood was found to rise 3 to 7.5 (average 6.4) times (table 1). Following

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the extirpation of a lobe of liver, therefore, the remainder of the organ releases vitamin A into the blood stream.

The mobilization of sugar from its reserves in the liver under similar circumstances is known to be associated with stimulation of the sympathico-adrenal system (Cannon, 1928). It may also be evoked by direct stimulation of the liver through the splanchnic (sympathetic) nerve, or indirectly by stimulation of the adrenal glands, or by the injection of adrenaline. Certain plasma proteins—e.g., fibrinogen—appear to be mobilized from stores in the liver by similar means (Foster and Whipple, 1922; Stübel, 1920; Riecker and Winters, 1931). In an attempt to define the stimuli for the discharge of vitamin A more closely, therefore, we turned to direct stimulation of the sympathico-adrenal system.

TABLE 1

Vitamin A and carotene concentrations of liver and whole blood just before and 3 to 5 hours after the removal of a lobe of liver

Concentrations in micrograms (γ) per gram of fresh liver or per ml. of blood

RABBIT	LIVER				BLOOD VITAMIN A	
	Vitamin A		Carotene			
	Before	After	Before	After	Before	After
1	42.9	29.7	0.036	0.023		
2	34.1	31.0	0.154	0.098	0.148	0.444
3	98.8	76.2	0.105	0.049	0.296	2.22
4	42.9	32.7	0.148	0.040	0.203	1.48
Averages.......	54.7	42.4	0.111	0.053	0.216	1.38

Electrical stimulation of the greater splanchnic nerve in the anesthetized rabbit with liver intact caused an increase in blood vitamin A of about 85 per cent (table 2).[2] Similar stimulation of the middle cervical sympathetic ganglion, which innervates primarily structures in the head, produced no appreciable effect on blood vitamin A (table 2). The mobilization of vitamin A through nerve action appears to depend upon direct stimulation of the abdominal viscera, probably primarily of the liver itself, and, as the following experiments show, of the adrenals.

The vitamin A content of the blood was found to be unchanged just before and 30 minutes after the intravenous injection of 1 ml. of normal saline solution. When in an otherwise identical procedure in the same rabbit the saline solution was replaced with 1 ml. of 1:1000 adrenaline,

[2] The nerve was stimulated with rapidly interrupted induction shocks set just too weak to cause contraction in nearby muscles. Ten seconds of stimulation were alternated with 10 seconds of rest for a total stimulation period of 2 minutes.

the blood vitamin A rose 75 per cent (table 2, rabbit 6). In rabbit 5 the injection of adrenaline yielded a much greater rise in blood vitamin A (about 170 per cent) than had stimulation of the splanchnic nerve. In general, following injection of 1 ml. of 1:1000 adrenaline, the blood vitamin A approximately doubled within 30 minutes, then slowly declined. In one animal (no. 6) tested four hours after the injection it still had not returned to the basal level.

Vitamin A is mobilized from its reserves in the liver, therefore, by a pattern of stimuli comparable with those which mobilize sugar and apparently certain plasma proteins.[3] All these phenomena form part of the sympathico-adrenal syndrome, most components of which play clearly recognizable rôles in the maintenance of the organism (Cannon, 1939).

TABLE 2

Vitamin A concentrations in micrograms per ml. of whole blood, just before and at various times after the procedures indicated

TREATMENT	RABBIT	BLOOD VITAMIN A				
		Before treatment	After treatment (minutes)			
			10	30	90	240
Stimulation of splanchnic nerve.......	5	0.252	0.466			
Stimulation of cervical ganglion.......	6	0.166	0.163			
Injection of 1 ml. normal saline.......	6	0.174		0.178		
Injection of 1 ml. 1:1000 adrenaline...	6	0.176		0.307		0.229
Injection of 1 ml. 1:1000 adrenaline...	5	0.248		0.666		
Injection of 1 ml. 1:1000 adrenaline...	7	0.277		0.540		
Injection of 1 ml. 1:1000 adrenaline...	7	0.292		0.555		
Injection of 1 ml. 1:1000 adrenaline...	8	0.207	0.359	0.344	0.270	
Injection of 1 ml. 1:1000 adrenaline...	9	0.174	0.398			

Some significant rôle might possibly be assigned to the mobilization of vitamin A also, if more were known of its general cellular functions. As yet we understand reasonably clearly only the participation of this vitamin in the visual cycle of the rods, and associated with this the rise of night-blindness in vitamin A deficiency (Wald, Jeghers and Arminio, 1938). The curious possibility exists that the mobilization of vitamin A

[3] Clausen *et al.* (1940) have reported the mobilization of vitamin A in the blood of dogs after feeding or injection of ethyl alcohol. The average rise in serum vitamin A 1½ hours after the introduction of alcohol by stomach tube was 62 per cent; but the vitamin A continued to rise beyond this value, in some cases for as long as 48 hours. Some evidence is presented of a direct effect of alcohol on the liver; it is suggested also that traumatic effects on other tissues may contribute to the result. The relation of these to the present observations is still obscure.

might temporarily alleviate dietary night-blindness, and this if true should lend it something of the emergency character of other sympathico-adrenal responses.

METHODS. *Liver carotene and vitamin A.* Fresh liver was ground thoroughly in a mortar with an equal weight of anhydrous sodium sulphate. The mixture was dried under low pressure at about 40°C. for 2 hours. It was ground to a fine powder and Soxhletted with low boiling benzine (petroleum ether, boiling range 20°–40°C.); matters were so arranged that the solvent passed through the material about 24 times. The benzine was distilled off under reduced pressure. The oily residue was taken up in 5 ml. of 6 per cent KOH in methanol, and saponified at 55°C. for 45 minutes under a stream of nitrogen. The saponification mixture was cooled, diluted with water to a methanol content of 65 per cent, and was shaken with 5 successive portions of benzine. The combined extracts were reduced to a volume of about 10 ml. and were partitioned in the following way: the benzine solution was shaken twice with equal volumes of 90 per cent methanol, then the combined methanol solutions were re-shaken once with fresh benzine. The benzine fraction was washed with water, evaporated to dryness, and taken up in chloroform for carotene determination. Several solutions prepared in this way have been checked spectrophotometrically; they possess the familiar absorption spectrum of carotene.

The methanol fraction was diluted with water to a methanol content of 65 per cent, and was extracted 5 times with benzine. The benzine was distilled off under suction, and the residue taken up in chloroform and combined with the benzine fraction (above) for the vitamin A determination. On mixing these solutions with antimony chloride reagent a clear blue color results, due to the single sharp band maximal at 620 mμ which is specific for vitamin A.

Blood vitamin A. For each test approximately 10 ml. of blood were drawn from an ear vein into a beaker containing a few crystals of potassium oxalate. To the whole blood was added three times its volume of 98 per cent methanol, and the mixture was centrifuged. The supernatant solution was decanted, brought to a methanol content of 65 per cent, and was extracted three times by shaking with benzine. The precipitate was shaken separately with 5 successive portions of benzine. All extracts were combined, washed with water, and the benzine distilled off under suction. The residue was taken up in chloroform for vitamin A determination.

Vitamin A and carotene concentrations. These were determined with the Zeiss Pulfrich Photometer, the use of which for such measurements was described several years ago by Wald (1935–36).

Vitamin A was determined by the following procedure. Three-tenths

milliliter of the chloroform solution to be tested was pipetted into a 10 mm. absorption cell and placed in position in the instrument. To this, 3.2 ml. of saturated antimony trichloride solution in chloroform were added, and the transmission of the resultant blue mixture was read within 12 to 15 seconds, through the S61 filter. This filter transmits a narrow band of wavelengths which coincides closely with the antimony chloride band of vitamin A. From the transmission, I/I_0, in which I_0 is the incident and I the transmitted intensity, the optical density or extinction, log (I_0/I) is computed. This is directly proportional to the vitamin A concentration, in accordance with Beer's Law. The optical density multiplied by 37 yields directly the concentration of vitamin A in micrograms per milliliter of the original solution, before the addition of the antimony chloride reagent.

The factor 37 was obtained through measurement by the present method of a large number of vitamin A preparations whose direct absorptions at 328 mμ were already known (Wald, unpublished observations). This factor was computed on the basis that the density at 328 mμ of a 1 per cent chloroform solution of pure vitamin A, 10 mm. in depth, is 1600.

Carotene concentrations were determined directly by measuring the densities of chloroform solutions through the S50 filter of the instrument. These solutions also obey Beer's Law over the range of the measurements. The method was calibrated with standard chloroform solutions of crystalline carotene (about 90 per cent β and 10 per cent α isomer). It was found that the density of a 10 mm. layer multiplied by 7.0 yields the concentration of carotene in micrograms per milliliter.

SUMMARY

The extirpation of a lobe of liver in the rabbit results in decreases of about 25 per cent in the vitamin A content and 50 per cent in the carotene content of the remainder of the organ. Simultaneously the blood vitamin A rises 3 to 7.5 times.

Electrical stimulation of the splanchnic nerve also results in a large increase in blood vitamin A. Similar stimulation of the cervical sympathetics is without effect.

Following the intravenous injection of 1 ml. of 1:1000 adrenaline, blood vitamin A is approximately doubled within 30 minutes, then slowly falls.

Vitamin A is mobilized from its reserves in the liver, therefore, by sympathico-adrenal stimulation comparable with that which mobilizes sugar and certain plasma proteins.

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OBSERVATIONS ON DENERVATED MUSCLE IN RELATION TO MYOTONIA

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Myotonia is a muscular defect, practically always inherited, in which stimulated muscles remain contracted for an abnormally long time after the stimulus has ceased. Striking similarities in the properties of denervated and myotonic muscles suggested that an investigation of denervated muscle might throw some light on the nature of myotonia.

METHOD. Cats under dial (Ciba, 0.75 cc. per kgm., intraperitonally) were used. The quadriceps muscle was examined in most experiments; a few experiments were performed on the gastrocnemius-soleus. The femoral, or sciatic, nerve was sectioned aseptically 7 to 24 days before the experiment. The femur or tibia was firmly fixed by means of drills, and the muscle tendons attached to the short end of a writing lever pulling against rubber bands. For direct stimulation steel needles were inserted into the body and tendon of the muscles. Condenser discharges were used most commonly for stimulation; in a few experiments a multivibrator circuit was employed.

As a rule drugs were injected into the aorta by retrograde cannulation of the inferior mesenteric artery. The adrenals were routinely ligated.

For recording the action currents of the spontaneous fibrillary activity, needles were inserted into the muscle and the potentials, after amplification, were observed and photographed in a cathode-ray oscillograph.

RESULTS. A. *Prolongation of the first contraction after a period of rest.* 1. Description. If a muscle which has been denervated for a week or more is stimulated electrically with a single shock, such as from a condenser, the first contraction after a period of rest almost always shows a more prolonged relaxation than succeeding contractions. An initial phase of fairly rapid relaxation changes to a phase of slow relaxation which may last 5 to 15 seconds or longer (fig. 1). In the more marked instances the rapid phase may be short, and most of relaxation occurs slowly. The change from the rapid to the slow phase is frequently abrupt (fig. 1). The extent of the prolongation of the contraction varies from animal to animal; the

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factors causing this variation have not been determined, but they do not appear to be related to the length of denervation.

If the muscle is stimulated again immediately after it has relaxed the second contraction shows little or none of the slow phase of relaxation. The same is true of succeeding contractions. If, however, the muscle is permitted to rest for 2 or 3 minutes and then stimulated, the first contraction again shows the phase of slow relaxation, equal to its original value; the prolonged contraction, therefore, can be quite accurately duplicated at 3-minute intervals. In some animals the phase of slow relaxation is marked at the beginning of an experiment but decreases gradually in spite of long rest periods.

If the muscle is stimulated repeatedly before the slow phase of relaxation is completed, the resulting contractions are superimposed on the slow

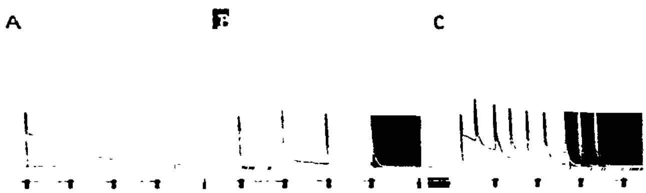

Fig. 1

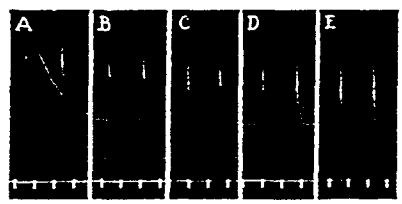

Fig. 2

Fig. 1. Prolongation of first contraction after a period of rest. Quadriceps denervated 16 days. Condenser shocks. A, B and C were taken at 3-minute intervals. Time in 5-second intervals. In A, the muscle was stimulated only once; in B, 4 times at 5-second intervals; and in C, 9 times at 2-second intervals.

Fig. 2. Increase in rapidity of relaxation on continued stimulation. Quadriceps denervated 7 days. Shocks from multivibrator circuit at about 9-second intervals. Time: 5-second intervals. A, first and second responses. B, 15th and 16th responses. C, 30 and 31st responses. D, 45th and 46th responses. E, 65th and 66th responses.

relaxation with little change in the elevated baseline (fig. 1). Occasionally the second contraction appears to cause a little drop in the baseline but none of the contractions raises it.

Although the markedly prolonged relaxation occurs only with the first contraction, relaxation of the second and succeeding contractions is slower than normal. Continued stimulation at 3- to 5-second intervals often produces a gradual increase in the rate of relaxation (fig. 2).

Rosenblueth and Luco (1937) noted that the prolonged contraction-remainders following electrical stimulation of denervated muscles were associated with action potentials. This would indicate that they were propagated contractions rather than contractures. The possibility, however, that some component of the contraction-remainder is due to contracture is not ruled out.

2. *Action of adrenaline.* Since stimulations at 3-minute intervals

elicit the slow phase of relaxation to about the same degree (fig. 1), the effects of drugs and various procedures on this phase can readily be determined. If the quadriceps muscle is tested at 3-minute intervals and the animal is then given an injection of adrenaline, subsequent tests for a period of 15 or more minutes show a marked increase in the speed of relaxation. This result was always obtained in the quadriceps muscle (fig. 3). Slowness of relaxation returns for the most part in 15 to 20 minutes and at

Fig. 3

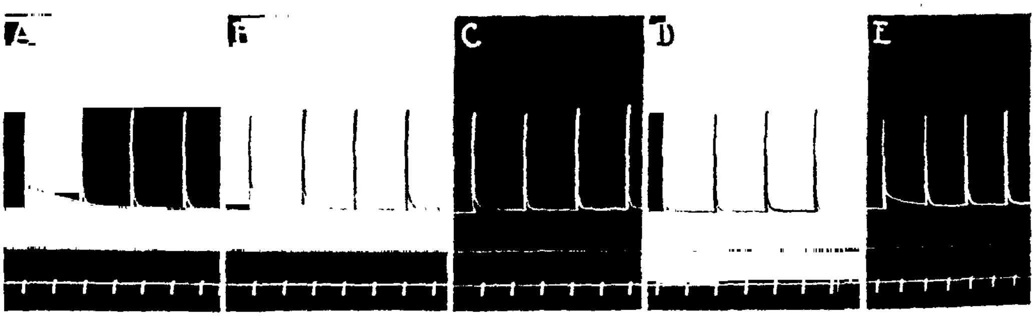

Fig. 4

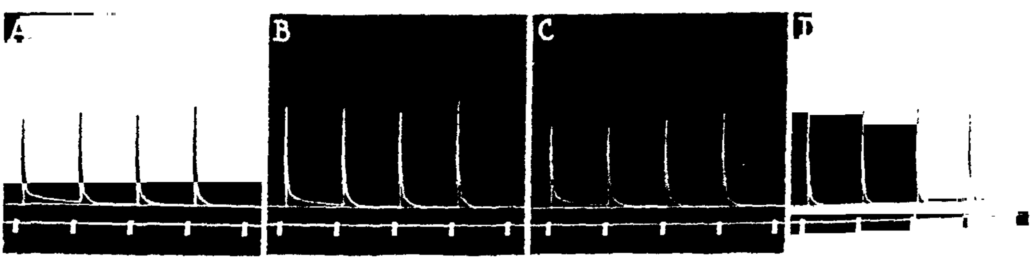

Fig. 3. Effect of adrenaline on the prolonged first contraction. Quadriceps, denervated 24 days. Adrenals ligated. Shocks from multivibrator circuit at approximately 9-second intervals. Series of 4 contractions were made at 3-minute intervals. Time: 5-second intervals. A and B, 2 series made before giving adrenaline. C, 30 seconds after giving 25 γ of adrenaline intra-arterially. D, 3.5 minutes after C. E, 22 minutes after D.

Fig. 4. Effect of circulatory arrest compared with the effect of adrenaline on the prolonged first contraction. Quadriceps, denervated 14 days. Adrenals ligated. Condenser shocks. Series of 4 contractions (at 5-second intervals) were made at 3-minute intervals. Time: 5-second intervals. The circulation to the muscle was stopped by clamping the abdominal aorta from just before series A until just after series B. Series C was recorded after restoring the circulation and just before giving adrenaline; series D, 2 minutes after injecting 25 γ of adrenaline into the jugular vein.

times there is a slight but definite accentuation of the slow relaxation after 25 to 30 minutes. These effects can be seen following the injection of 25 γ of adrenaline into the jugular vein.

The possibility suggests itself that the action of adrenaline is on the blood supply rather than directly on the muscle. It can be shown, however, that whereas an injection of adrenaline produces a marked effect within 2 minutes, stopping the circulation to the muscle for 3 minutes de-

creases the length of the contraction little if at all (fig. 4). The conclusion that adrenaline acts mainly or entirely on the muscle seems justified.

The effect of adrenaline on the gastrocnemius-soleus muscles is quantitatively different from that on the quadriceps. An injection of adrenaline is followed by an increased speed of relaxation occurring within 2 to 4 minutes after the injection, but the improvement is much less than in the quadriceps. A brief period of improvement is followed by a longer period during which the slow phase of relaxation gradually increases, reaches a maximum in 15 to 19 minutes, and then gradually returns to its original extent (fig. 5). In this case, then, an initial decrease in the relaxation rate is of small degree and short duration, whereas the secondary increase in the relaxation rate is marked and prolonged.

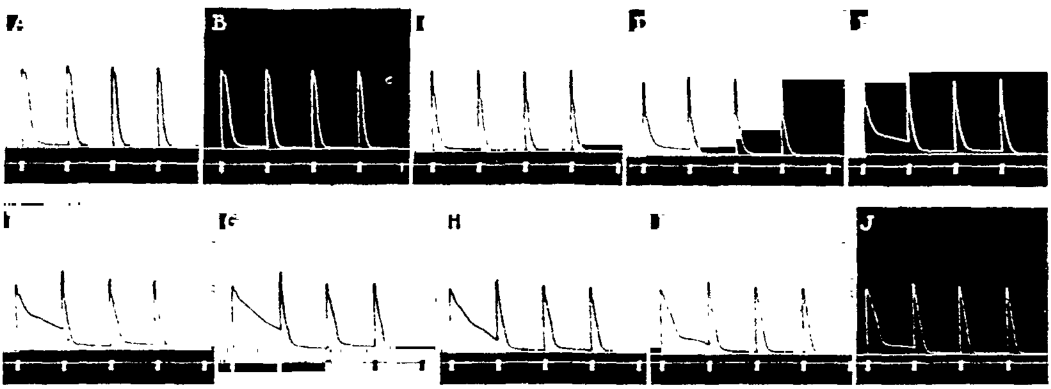

Fig. 5. Effect of adrenaline on the prolonged first contraction. Gastrocnemius-soleus, denervated 15 days. Condenser shocks. Adrenals ligated. Series of 4 contractions made at 3-minute intervals. Time: 5-second intervals. A and B, taken before giving adrenaline. One minute before C, 25 γ of adrenaline injected into aorta. C to J, show successive series at 3-minute intervals.

3. Action of potassium. Potassium chloride (5–30 mgm.) injected intra-arterially 20 to 30 seconds before a test produces a marked decrease in the duration of the contractions (fig. 6). The effect is evident for only 2 or 3 tests (at 3-min. intervals) and occurs in both quadriceps and gastrocnemius-soleus muscles with doses of wide range. No secondary phase of accentuation is seen.

4. Action of quinine. Quinine markedly decreases the duration of the contractions; both the rapid and slow phases of relaxation are involved (figs. 7 and 8). An increase in extent of contraction also is usually evident (fig. 7).

5. Action of calcium. Calcium chloride (15 mgm.) injected intra-arterially decreases the duration of the contractions (fig. 9). It produces also a decrease in the extent of contraction. As the effect wears off the duration and extent of the contractions increase.

6. Action of eserine and prostigmin. No clear-cut effect of eserine or

Fig. 6

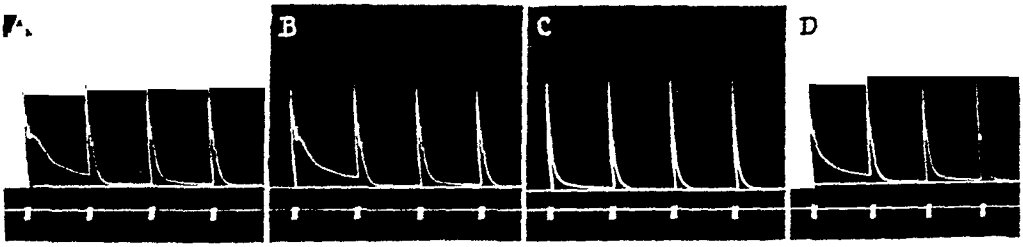

Fig. 7

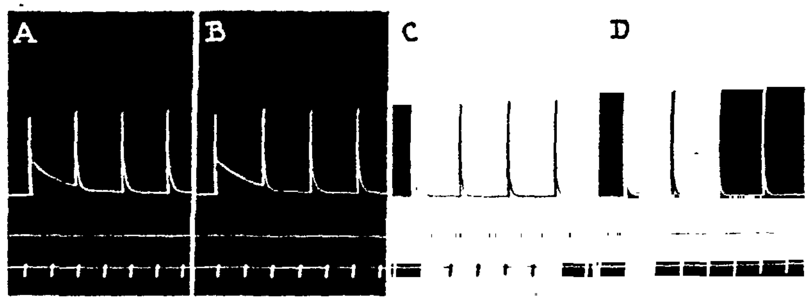

Fig. 8

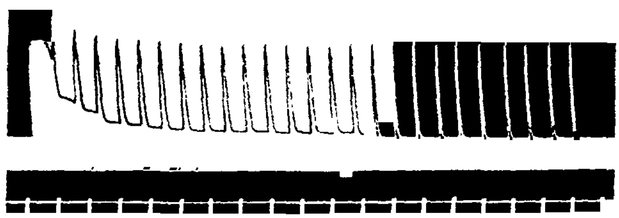

Fig. 9

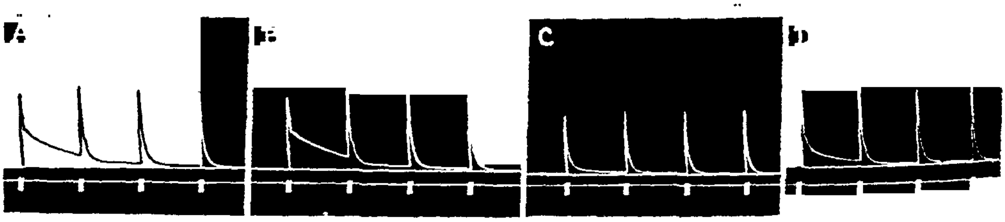

Fig. 6. Effect of potassium chloride on the prolonged contractions. Quadriceps, denervated 16 days. Adrenals ligated. Condenser shocks. Series of 4 contractions were taken at 3-minute intervals. Time: 5-second intervals. A and B, before giving potassium chloride. C, 30 seconds after injecting 15 mgm. of potassium chloride into aorta. D, 3 minutes after C.

Fig. 7. Effect of quinine on the prolonged contractions. Quadriceps, denervated 24 days. Adrenals ligated. Stimulation by multivibrator circuit at approximately 9-second intervals. Series of 4 contractions at 3-minute intervals. Time: 5-second intervals. A and B, before giving quinine. C, 2.5 minutes after injection of quinine hydrochloride (15 mgm.) into aorta. D, 8.5 minutes after C.

Fig. 8. Effect of quinine on the contractions of denervated muscle. Quadriceps, denervated 7 days. Stimulation by multivibrator circuit at approximately 3-second intervals. Time: 5-second intervals. At signal 15 mgm. of quinine hydrochloride injected into aorta.

Fig. 9. Effect of calcium chloride on the prolonged first contraction. Quadriceps, denervated 16 days. Condenser shocks. Series of 4 contractions at 3-minute intervals. Time: 5-second intervals. A and B, before giving calcium. C, 0.5 minute after injecting calcium chloride (15 mgm.) into aorta. D, 18 minutes after C.

prostigmin could be shown. In 2 experiments a questionable increase in duration of relaxation was seen. With prostigmin small doses produced no effect; larger doses produced a shortening of the muscle which interfered with further tests.

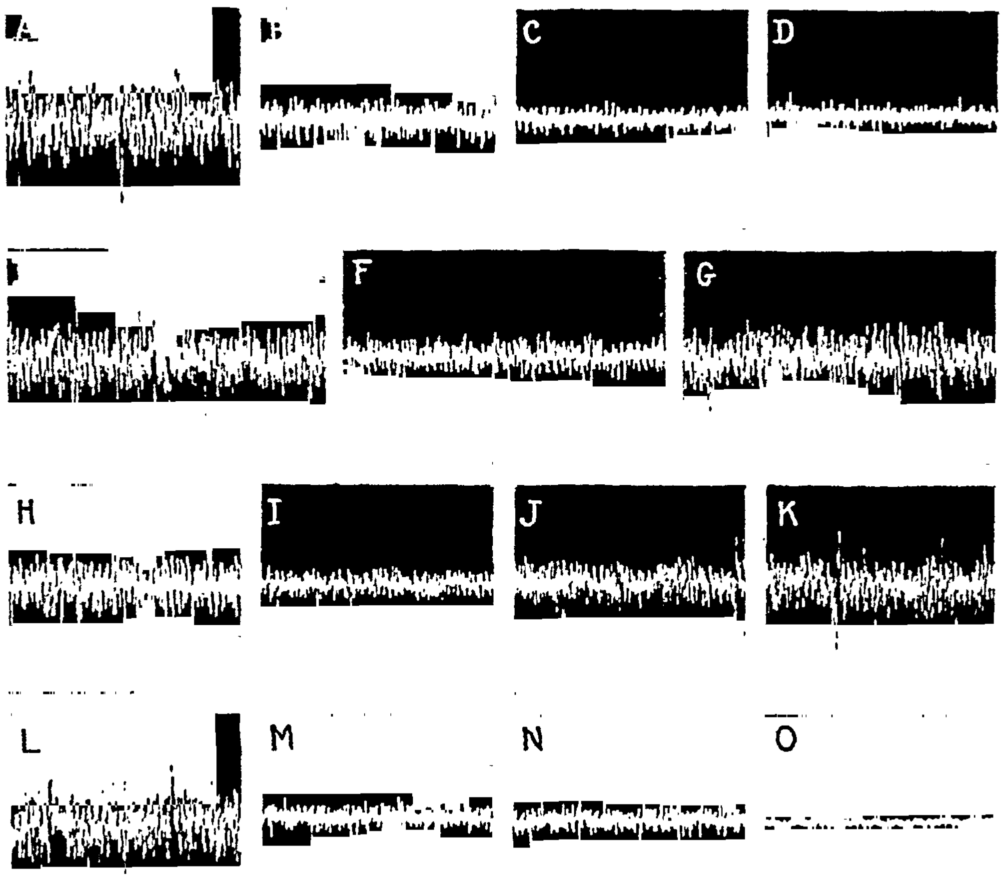

Fig. 10. Effect of various drugs on the spontaneous fibrillation of denervated muscle. Quadriceps, denervated 12 days. Adrenals ligated. A, background activity. B, 1.5 minutes after injection of 40 γ of adrenaline into aorta. C, 2 minutes after B. D, 1 minute after C. E, background activity. F, 3 minutes after stopping circulation to muscle by clamping aorta. G, 1 minute after restoring circulation. H, background activity. I, 30 seconds after injecting 20 mgm. of potassium chloride into aorta. J, 30 seconds after I. K, 30 seconds after J. L, background activity. M, 1 minute after injection of 20 mgm. of quinine hydrochloride into aorta. N, 7 minutes after M. O, control record from normal muscle of opposite side.

B. *Spontaneous fibrillary activity.* Within a week after denervation the muscles of a cat show spontaneous fibrillations, manifested electrically as continuous irregular asynchronous spike potentials (fig. 10). The effect of adrenaline on this activity was studied in 2 animals; in addition, the action of potassium, quinine hydrochloride and interruption of the circulation was

studied in 1 of these animals. In 1 animal 2 injections of 25 γ of adrenaline into the aorta resulted in a temporary (1 to 2 min.) increase in spike potentials followed by a marked decrease which lasted for 2 to 3 minutes, and then a gradual return to normal. In another animal 25 γ of adrenaline produced only the brief increase of spike potentials; 40 γ given shortly afterwards elicited only a marked decrease of the potentials (fig. 10). The decrease at the end of 3.5 minutes was more marked than that obtained by completely cutting off the circulation to the muscle for 3 minutes (fig. 10).

An intra-arterial injection of 15 mgm. of potassium chloride produced a striking but brief (1 to 2 min.) decrease of spike potentials (fig. 10).

An injection of 15 mgm. of quinine hydrochloride produced a decrease of the spikes which was evident in 15 seconds and still present after 8 minutes (fig. 10).

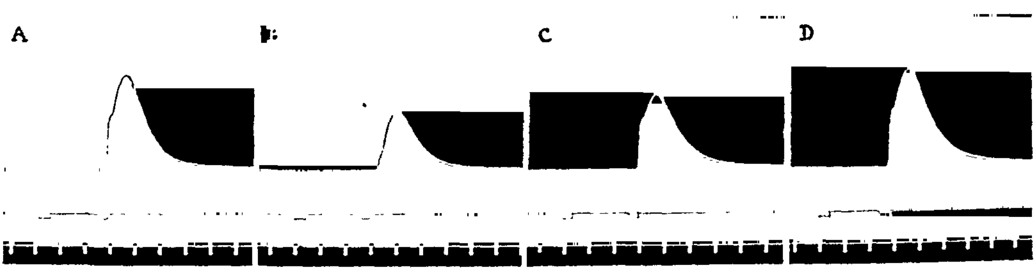

Fig. 11. Effect of potassium chloride on the response of denervated muscle to acetylcholine. Quadriceps, denervated 16 days. Adrenals ligated. Time: 5-second intervals. A, B, C and D were taken at 5-minute intervals. At second signal in each instance 10 γ of acetylcholine injected intra-arterially. At first signal in A, C, and D, 0.3 cc. of mammalian Ringer injected intra-arterially. At the first signal in B, 7.5 mgm. of potassium chloride in 0.3 cc. of distilled water injected intra-arterially.

C. *Contracture produced by acetylcholine.* 1. Action of quinine. Quinine decreases the response (contracture and contraction) of denervated muscle to acetylcholine, an observation previously made by Harvey (1939) and Oester and Maaske (1939).

2. Action of potassium. An intra-arterial injection of potassium chloride, given shortly before the injection of acetylcholine, decreases the response of the muscle to acetylcholine (fig. 11). This occurs both when the potassium chloride alone is not sufficient to cause an evident contraction and also when it causes a contraction. The response of the muscle to small doses of acetylcholine may be almost completely inhibited. The effect of potassium is gone in 5 to 15 minutes. In 1 animal it decreased both the contraction and the contracture responses to acetylcholine; although the contraction appeared more affected, the contracture took longer to return to normal (fig. 11).

Discussion. A few days after being denervated, mammalian skeletal

muscle shows, among other changes, 3 new phenomena: 1, a prolonged first contraction after a period of rest; 2, a spontaneous fibrillary activity; and 3, an increased sensitivity to acetylcholine and an increased tendency to respond to acetylcholine with contracture. These phenomena show a striking uniformity in response to several drugs. Adrenaline given shortly before acetylcholine will usually inhibit the response of the denervated muscle to acetylcholine, but under special conditions it may accentuate the response (Luco, 1939). Similarly, adrenaline usually inhibits the prolonged first contraction and the spontaneous fibrillation of denervated muscle, though at times it may have opposite effects. Potassium chloride injected intra-arterially inhibits all three phenomena. Quinine likewise inhibits all three phenomena. Frank, Nothmann and Guttmann (1933) found that calcium inhibits the response of denervated muscle to acetylcholine, and Langley (1915-16) reported that calcium inhibits the spontaneous fibrillations of denervated muscle. Calcium has been shown above to inhibit the prolonged first contraction. The response of denervated muscle to acetylcholine is increased by eserine as is the spontaneous fibrillary activity (Rosenblueth and Luco, 1937). The evidence that eserine produces an increase in the prolonged first contraction is not conclusive. Such uniformity of three phenomena which are produced by the same procedure strongly suggests that they represent different manifestations of the same change in the muscle.

The denervated muscle and the myotonic muscle show remarkable similarities (cf. table 1). The most remarkable is the occurrence of the prolonged contraction following a period of rest. This is seen in the myotonic muscle after contractions produced by nerve impulses or by mechanical or electrical stimuli. In the denervated muscle this prolonged first contraction occurs after electrical stimulation; whether it occurs after mechanical stimulation has not been determined. The prolonged contraction seen in denervated muscle is influenced by several drugs in almost exactly the same manner as the myotonic contraction. Adrenaline, calcium and quinine influence both responses in the same manner. Potassium at first glance appears to produce different effects, but in tests on denervated muscle the drug was injected intra-arterially, whereas in observations on myotonia it was given by mouth. Possibly the action of potassium chloride given by mouth over long periods of time would differ from its action when given intra-arterially. Prostigmin usually increases myotonia but no definite effect was shown in the denervated muscle. Brown and Harvey (1939), however, were unable to show that the muscles of goats with a condition which appears analogous to myotonia were more sensitive to eserine than normal muscles. The occurrence of the prolonged first contraction which behaves so much like the myotonic contraction and the numerous other points of similarity in the properties of

TABLE 1

	DENERVATED MUSCLE (TOWER, 1939)	MYOTONIA (RAVIN, 1939)
Mechanical excitability	Increased	Increased
Response to galvanic current	Increased excitability ACC equal to or greater than CCC Sustained contraction during passage of current	Increased excitability ACC often equal to or greater than CCC Sustained contraction during passage of current
Response to faradic current	Diminished excitability	(Cannot distinguish nerve from muscle excitation)
Spontaneous fibrillation	Present	Probably not usually present
Sensitivity to acetylcholine	Increased	Increased. Also reported as normal (Brown and Harvey, 1939)
Sensitivity to potassium chloride	Increased	Increased (Brown and Harvey, 1939)
Prolongation of first contraction after a period of rest	Seen following electrical stimulation	Seen following all types of stimulation
Effect on prolonged contraction of:		
1. Adrenaline	Decreases. May increase	Decreases. May increase
2. Quinine	Decreases	Decreases
3. Calcium	Decreases	Decreases
4. Potassium	Decreases on intra-arterial injection	Increases on oral administration
5. Eserine	No definite effect	Increases. No change also reported (Brown and Harvey, 1939)
Histology	Increase in sarcolemma nuclei and migration of nuclei to interior of fiber Atrophy	Increase in sarcolemma nuclei. Occurrence of nuclei in interior of fiber also reported No atrophy in pure myotonia but atrophy is commonly associated with myotonia in dystrophia myotonica

the denervated and the myotonic muscle make it highly probable that one of the results of denervation is to produce a condition similar to that found in the myotonic muscle.

In a previous article (Ravin, 1940), myotonia was ascribed to a defect in the muscle rather than in the neuromuscular transmission. This conclusion was based on two considerations: 1, the similarity of myotonia and a group of representative contractures, and 2, an analysis of that characteristic of myotonia manifest in a duration of the myotonic contraction independent of the associated voluntary contraction. (Note the analogy between this characteristic and the independence of the prolonged first contraction of denervated muscle from subsequently induced contractions.) To this evidence for a muscular location of the defect in myotonia is now added the further evidence of 1, the similarity of the myotonic and denervated muscle, and 2, the observations of Brown and Harvey (1939) on goats, having a condition which appears to be the counterpart of myotonia of man, that the defect persists after curarization and denervation.

If myotonia is due to a defect in the muscle, is it a contracture or a contraction? It would seem that all that is necessary to settle this point is to determine the presence or absence of spike potentials. The problem is not simple: if no spikes are found, the instruments may be insensitive or the technique wrong. If spike potentials are found, they may be due to associated twitching, and the presence of a contracture is still not ruled out. Both the presence and the absence of action currents in myotonia have been reported recently. Brown and Harvey (1939) found them associated with the prolonged contractions occurring in their goats. On the other hand, Eichler and Hattingberg (1938) were unable to find them always in myotonic contractions. Brown and Harvey state that when the recording electrodes are applied so as to lead from a large proportion of the muscle fibers (as was done by Eichler and Hattingberg) the action currents may not be found during myotonic contractions, although a more localized lead, such as a concentric needle electrode, may reveal their presence. Lindsley and Curnen (1936) used needle electrodes in their studies on patients with myotonia and found action currents present. The conclusion appears safe that myotonic contractions are associated with action currents. It is not yet safe to exclude entirely the possibility of the occurrence also of a contracture.

The myotonic contraction behaves in many respects like the members of a group of representative contractures (Ravin, 1940). This observation presents three possibilities: 1, myotonia is a contracture; 2, myotonia is a contraction which, however, shows properties similar to a contracture; and 3, myotonia is a combination of a contraction and a contracture. The first possibility, that myotonia is a pure contracture, seems ruled out by

the work of Brown and Harvey, and Lindsley and Curnen. The third possibility requires, like the second, that the portion of myotonia which is a contraction should show many of the properties of the contracture. This is just what was found in the denervated muscle. The denervated muscle displayed a prolonged contraction, associated with action potentials, which behaved in many ways like the acetylcholine contracture. There is, then, an additional reason for believing that myotonic muscle and denervated muscle have some change in common. This change results in 1, prolonged contractions associated with action currents, and 2, increased tendency to respond by contracture. Since both responses behave in like manner to several drugs and conditions, the similarities which myotonia shows to contractures are easily understood, even if it is concluded that a contracture is not ordinarily present. It is furthermore not unlikely that some portion of the myotonic response may occasionally be a contracture. The question of whether myotonia is a tetanus or a contracture has lost much of its significance.

The ability of adrenaline to inhibit acetylcholine contractures has been attributed to a vasoconstriction which prevents the acetylcholine from reaching the muscle fibers (Dale and Gaddum, 1930). The action of adrenaline on the prolonged first contraction of denervated muscle furnishes evidence that it does act upon the muscle itself. Therefore, although the vasoconstrictor effect of adrenaline may play a part in checking the action of acetylcholine, there seems to be very little question that part of the action of adrenaline is on the muscle itself.

The difference in response of the quadriceps and gastrocnemius muscles to adrenaline may perhaps be the explanation of a discrepancy in observations on myotonia. In tests made mainly on the small muscles of the thumb, the author found that adrenaline always produced a marked decrease in myotonia (Ravin, 1940). This result differed from that reported by several previous observers. At the time no explanation could be offered; now it seems possible that in some muscles of myotonic patients the main effect of adrenaline is aggravation of the myotonia and that a fleeting improvement may be easily overlooked.

SUMMARY

If a denervated cat's muscle is stimulated electrically with single shocks, as from a condenser, the first contraction after a period of rest is more prolonged than succeeding contractions (figs. 1 and 2). The characteristics and the reactions to drugs of this prolonged contraction have been studied.

Adrenaline may decrease or increase the duration of the contraction (figs. 3–5). Potassium chloride (fig. 6), quinine hydrochloride (figs. 7 and 8), and calcium chloride (fig. 9) decrease its duration.

The spontaneous fibrillary activity characteristic of denervated muscle is decreased by intra-arterial injections of quinine hydrochloride and potassium chloride (fig. 10). Adrenaline may produce either an increase or a decrease of the fibrillations (fig. 10).

The response of denervated muscle to acetylcholine is decreased by quinine and potassium chloride (fig. 11).

That various drugs induce uniform effects on three of the characteristics of denervated muscle—spontaneous fibrillation, prolonged first contraction and changed response to acetylcholine—is emphasized.

The marked similarities in the characteristics of denervated muscle and myotonic muscle are pointed out, and the suggestion is made that one of the results of denervation is to produce a change in muscle similar to that which is present in myotonia.

I am indebted to Dr. W. B. Cannon for many helpful suggestions, and to Dr. A. Rosenblueth for taking the electrical records.

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EFFECTS OF QUININE ON MAMMALIAN SKELETAL MUSCLE

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The discovery by Wolf (1936) of the remarkable effect of quinine on myotonia has renewed interest in the actions of quinine on skeletal muscle. Harvey (1939) and Oester and Maaske (1939) investigated the problem with essentially similar results. The following important actions of quinine were observed: 1, an increase in the height of response to single maximal stimuli; 2, a depression of the response to tetanic stimulation; 3, an increase in the refractory period of the muscle; 4, a curare-like action; 5, an inhibition of the eserine-produced potentiation of muscle twitches, and 6, a depression of the response of denervated muscle to acetylcholine. Certain phases of the action of quinine seemed worthy of further investigation; many of the results here reported confirm and extend previously known actions of quinine.

METHODS. Cats were used in all experiments except those on the atrophy of denervated muscle. The anesthetic was dial (Ciba), 0.75 cc. per kgm., intraperitoneally. The quadriceps muscle was used for most experiments; a few performed on the gastrocnemius-soleus and tibialis anticus yielded similar results. The femur, or tibia, was fixed by means of drills, and the muscle tendons attached to the short end of a writing lever pulling against rubber bands. The femoral nerve was cut or crushed and stimulated distally by shielded silver electrodes. For direct stimulation of the muscle steel needles were inserted into the body and tendon. Rectangular waves from a multivibrator circuit were most frequently used for stimulation; the output was usually passed through a transformer before being applied to the nerves. Condenser discharges of various capacities and a thyratron circuit were also occasionally used for stimlation. All stimuli were maximal.

All injections of quinine were made into the abdominal aorta by retrograde cannulation of the inferior mesenteric artery. To increase the concentration of the drug going to the muscle the opposite iliac artery and the caudal branches of the aorta were ligated. The drugs employed were quinine hydrochloride, physostigmine salicylate, prostigmin (Roche), atropine sulfate, acetylcholine chloride (Merck), and curare (the Brazilian

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crude product). The quinine hydrochloride was dissolved in warm distilled water to make a 5 per cent solution.

White rats were used for the study of the atrophy of denervated muscles. In each animal the gastrocnemius-soleus muscle on one side was denervated by removal of a segment of the sciatic nerve. In both control and treated animals an equal number of right-sided and left-sided denervations were made. Half the animals received quinine injections; the other half were given injections of saline and served as controls. A 2.5 to 5.0 per cent solution of quinine hydrochloride was given subcutaneously. Aside from frequent local reactions most of the animals showed no ill effects. In one group of animals a fairly large dose of quinine hydrochloride was used and an occasional death shortly after the injection indicated that the dose was close to lethal. Since loss of weight complicates the results (Hines and Knowlton, 1934–35) an attempt was made to avoid any changes of weight, and animals showing a definite weight loss were discarded. At the end of the test period the animals were killed with carbon monoxide and the muscles dissected out and kept in weighing bottles until weighed.

RESULTS. *Effect on indirectly stimulated muscle.* Rates of stimulation ranging from one every 30 seconds to 530 per second were investigated. The effect of quinine does not consist simple of an augmentation of the response to single stimuli and a depression of tetani. With small doses of quinine (5 to 15 mgm.) an increase in response for short periods (5–15 sec.) may be obtained with frequencies as high as 30 to 40 per second (figs. 1 and 3). At 60 per second the initial tension is usually unchanged or slightly increased by a small dose of quinine, but instead of being maintained the tension decreases as stimulation is continued. At rates of stimulation above 60 per second, even the initial tension may be lower than normal. The higher the rate of stimulation the more rapid the fall of tension as compared to normal. With larger doses of quinine, the response to high rates of stimulation may consist merely of a single twitch (fig. 4). If the muscle is stimulated at a rate sufficiently high (530 per sec.) to show the various stages of neuromuscular transmission (Rosenblueth and Cannon, 1940), it is evident that there is no selective augmentation or depression of any of the stages; from its initial height the tension falls more rapidly than normal and stages 3a and 3c are decreased in height or fail to appear (fig. 4). The action of an intra-arterial injection of quinine is at its maximum within 20 to 40 seconds and then gradually decreases so that most of the effect may be gone in 20 to 30 minutes.

Repeated doses of quinine further increase the response at slow rates of stimulation and at the same time lower the rate of stimulation required to show depression (figs. 1 and 3). With a sufficiently large dose of quinine (15 to 20 mgm. repeated 4 to 6 times at 8- to 10-min. intervals) the response to all rates of stimulation may be depressed even to the point of

extinction. The more frequent the rate of stimulation and the greater the dose of quinine the more evident the depression. When the response to nerve stimulation is depressed even at very slow rates of stimulation (1 every 15 sec.), it can be shown that the response of the muscle to direct stimulation is still much above the pre-quinine level (fig. 2). Moreover,

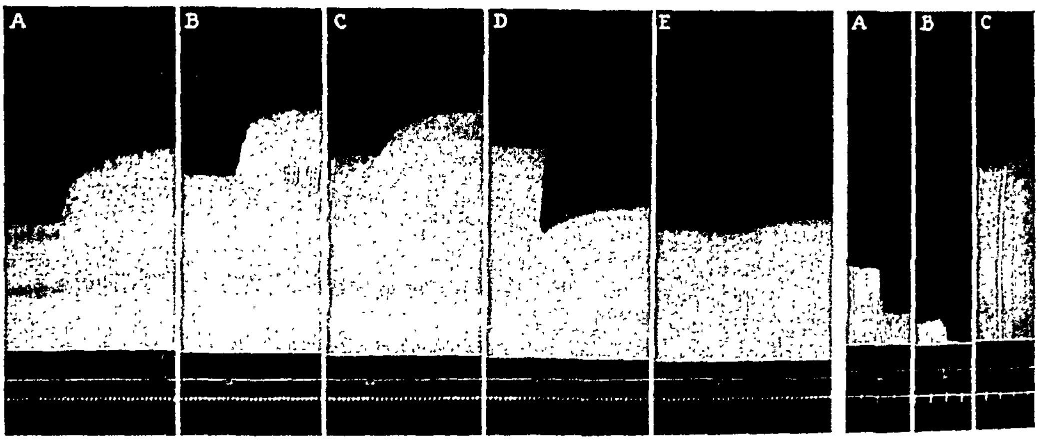

Fig. 1 Fig. 2

Fig. 1. Effect of quinine at slow rates of stimulation. Left quadriceps; femoral nerve stimulated at 2-second intervals by multivibrator circuit. Time: 5-sec. intervals.

A, 20 mgm. of quinine hydrochloride injected intra-arterially at signal.

B, 10 minutes later; 20 mgm. of quinine hydrochloride injected intra-arterially at signal.

C. 10 minutes after B; 20 mgm. quinine hydrochloride injected intra-arterially at signal.

D. 10 minutes after C; 20 mgm. quinine hydrochloride injected intra-arterially at signal.

E, 8 minutes after D; eserine (1 mgm. per kgm.) injected intra-arterially at signal.

Fig. 2. Effect of direct stimulation of muscle after quinine has markedly depressed response to indirect stimulation. Left quadriceps. Previous to A the animal had been given large doses of quinine; an initial increased contraction was followed by a decrease, and at A the responses are somewhat more than one-half the original height. Time: 30-sec. intervals.

A and B. Femoral nerve stimulated at approximately 5-sec. intervals. Quinine hydrocholoride (25 mgm. intra-arterially) at signal in A and (50 mgm. intra-arterially) at signal in B.

C. Three minutes after B. Direct stimulation of the muscle. Quinine hydrochloride (30 mgm. intra-arterially) at signal.

another injection of quinine will at times produce a further increase in the response (fig. 2). Very large doses are required to produce a depression of the response to slow, direct stimulation.

In some animals at slow rates of stimulation an injection of quinine may produce a brief decrease in response before augmentation.

At one stage in quinine action, rates of stimulation between 3 and 30 per

second may produce an increased initial tension, followed by a fairly rapid fall in tension and a slower rise (fig. 3C)—a phenomenon suggestive in some respects of the action of eserine. Also reminiscent of the action of eserine is the more complete tetanus obtained at slow rates of stimulation (30 per sec., fig. 3).

Harvey's observation that the increased response produced by quinine was not accompanied by any evidence of repetitiveness as judged by the action current was confirmed.

Each injection of quinine produced a marked fall of blood pressure followed by a gradual recovery. After an intra-arterial injection, however, the changes in the response of the muscle occurred before the blood-pressure changes and no correlation was evident between the two.

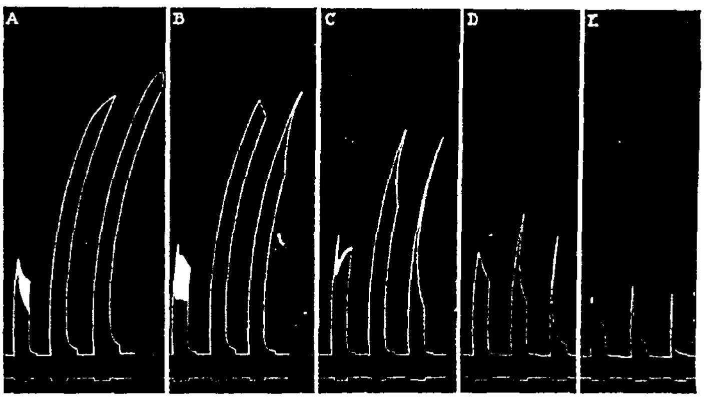

Fig. 3. Effect of quinine at intermediate rates of stimulation. Quadriceps; femoral nerve stimulated for 5 seconds every 2 minutes. Signal indicates duration of stimulation. Thyratron circuit. In each group the responses are to 30, 60 and 120 stimuli per second respectively.

A, before quinine. B, 1 minute after 15 mgm. of quinine hydrochloride. C, 4 minutes after B and 1 minute after 15 mgm. more of quinine hydrochloride. D, 4 minutes after C and 1 minute after 15 mgm. more of quinine hydrochloride. E, 4 minutes after D and 1 minute after another 15 mgm. of quinine hydrochloride.

Graham (1935) has shown that quinine produces changes in the excitability of nerves; therefore, the possibility that an action on the nerve might account for some of the changes seen on indirect stimulation must be considered. In one experiment the nerve potential showed no change in amplitude after injection of sufficient quinine to extinquish the muscular response. A more complete study is required, however, before the problem can be settled.

The quinine-eserine relationship in indirectly stimulated muscles. The response of a muscle after dosage with eserine will depend upon the frequency of stimulation. At slow rates (1 every 10 sec.) the response is usually increased; at frequencies of 3 to 6 per second the response is usually decreased; at higher frequencies the initial rise in tension is followed by a

prompt fall (Wedensky inhibition), then a slower rise, and finally a slow decline (plus-minus-plus response). Quinine given after eserine antagonizes these actions of eserine; it reduces the increase at slow rates of stimulation and the depression at higher rates of stimulation. If sufficient quinine is given the potentiation produced by quinine at slower rates of stimulation becomes evident (figs. 5 and 6).

If a small dose of quinine has been given, a sufficiently large dose of eserine (1 mgm. per kgm.) will produce an eserine potentiation at slow rates of stimulation. This can in turn be overcome by additional quinine.

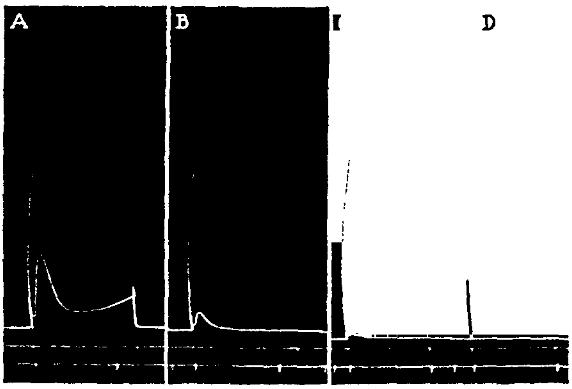

Fig. 4

Fig. 5

Fig. 4. Effect of quinine at high rates of stimulation. Quadriceps; stimulation of femoral nerve at 530 per second by multivibrator circuit. Lower signal, time in 1-minute intervals; upper signal, beginning and end of stimulation. A, before quinine. B, 1 minute after 15 mgm. of quinine hydrochloride. C, 40 minutes after B; two injections of 15 mgm. each of quinine hydrochloride had been given meanwhile. D, 5 minutes after C and 1 minute after another injection of 15 mgm. of quinine.

Fig. 5. Antagonism of quinine and eserine. Quadriceps; femoral nerve stimulated at approximately 10-sec. intervals with condenser shocks. Atropine (1 mgm. per kgm.). Time: 5-sec. intervals. At first signal eserine (1 mgm. per kgm.) was injected into the jugular vein. At second and third signals 10 mgm. of quinine hydrochloride were injected intraarterially. At fourth signal eserine (1 mgm. per kgm.) was again given.

After a certain amount of quinine has been given, however, the usual doses of eserine produce no effect, or may produce slight depression. An exception is seen in those instances where quinine is given to the point of producing depression at slow rates of stimulation. In these circumstances an injection of eserine, if it is the first the animal has received, will produce a small potentiation (fig. 1). The depression which quinine produces at high rates of stimulation is, however, little if at all improved by eserine.

Very large doses of eserine will depress somewhat the quinine-produced potentiation. A more evident depression is seen following prostigmin;

injection of a fairly large dose may prevent or inhibit potentiation by a subsequent, not too large, dose of quinine (fig. 7). It is difficult to demonstrate any such effect with eserine.

Effect on post-tetanic potentiation. After a short tetanization the response of a muscle to single maximal stimuli is increased. Thus, indirect stimulation of the quadriceps at a rate of 530 per second for 10 seconds produces potentiation to subsequent single shocks. This potentiation is inhibited by quinine (fig. 8). If not too much quinine is given the post-tetanic potentiation may be restored or increased beyond normal values by eserine and then again inhibited by more quinine. Following large doses of quinine eserine is no longer effective. With large doses of quinine

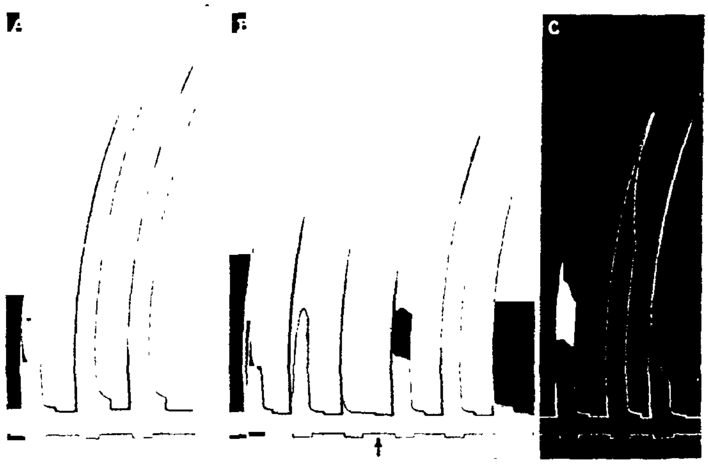

Fig. 6. Antagonism of quinine and eserine. Quadriceps; femoral nerve stimulated by thyratron for 5 seconds at 2-minute intervals. Signal indicates duration of stimulation. The order of stimulation was 30, 60 and 120 per second in each instance. A, before eserine. B, 10 minutes after giving eserine (0.3 mgm. per kgm. intravenously). At arrow quinine hydrochloride (15 mgm. intra-arterially). C, 8 minutes after B and just after another injection of quinine hydrochloride (15 mgm. intra-arterially).

also a secondary phase of depression of the twitches may occasionally be seen 1.5 to 2 minutes after the tetanus.

At the stage of quinine action where the response to nerve stimulation at slow rates is markedly depressed tetanic stimulation does not produce a potentiation of the response, such as occurs with curarized muscles. If, however, the muscle has rested for 20 to 30 minutes, the gradually improving response (due to decrease of quinine action) will show a post-tetanic increase.

Effect on curarized muscle. Previously reported observations (Harvey, 1939; Oester and Maaske, 1939), that quinine produces a potentiation in the response of a curarized muscle to slow rates of direct stimulation and that quinine further decreases the response of a partially curarized muscle to

nerve stimulation, are easily confirmed and can be predicted from what has been reported above. With small doses of quinine the increase in response at slow rates of stimulation is associated with a depression at more rapid rates. With increasing doses of quinine the depression occurs at lower frequencies and is finally seen at all frequencies. There are, however, two important differences from the indirectly stimulated muscle: 1, much

Fig. 7

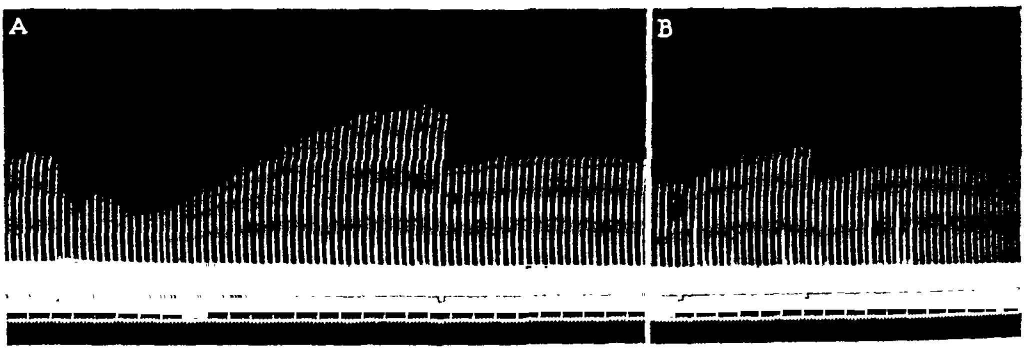

Fig. 8

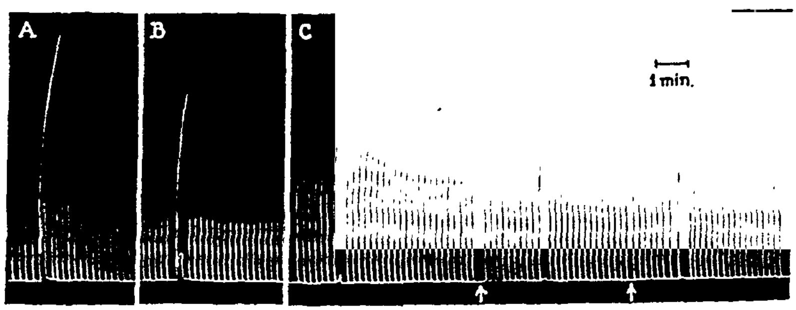

Fig. 7. Antagonism of quinine and prostigmin. Quadriceps; femoral nerve stimulated at 10-sec. intervals by condenser shocks. Atropine (1 mgm. per kgm.). Time: 5- and 30-sec. intervals. A, at first signal prostigmin (0.5 mgm.) was injected intra-arterially. At second signal quinine hydrochloride (20 mgm.) was injected intra-arterially. B, 22 minutes after A. At first signal quinine hydrochloride (20 mgm.) was injected intra-arterially and at second signal prostigmin (0.5 mgm.) was injected intra-arterially.

Fig. 8. Effect of quinine on post-tetanic potentiation of twitches. Quadriceps; femoral nerve stimulated every 10 seconds by multivibrator circuit. Atropine (1 mgm. per kgm.). Tetanic stimulation: 530 per second for 10 seconds. A, before quinine. B, 6.5 minutes after 10 mgm. of quinine hydrochloride. C, 20 minutes after B and 12 minutes after eserine (0.5 mgm. per kgm.). At arrows 10 mgm. of quinine hydrochloride were injected.

larger doses of quinine are required to produce the depression; and 2, the degree of depression obtained is not so marked (fig. 9).

Effects on denervated muscle. 1. Inhibition of spontaneous fibrillary activity. All denervated muscles show within several days after denervation a spontaneous fibrillation which is manifest electrically as a continuous irregular, asynchronous series of spike potentials. Quinine injected intra-arterially produces a marked inhibition of this fibrillary activity.

2. Effect on atrophy of denervated muscle. The marked atrophy which occurs in a denervated muscle has been attributed to its continuous fibrillary activity. If this is true, quinine might be expected to inhibit the atrophy. The results of a number of experiments carried out as indicated under "Methods" are given in table 1. It is evident that under the conditions of the experiment no obvious effect was noted.

3. Effect on the response to electrical stimulation. If a denervated muscle is stimulated with single shocks the first contraction following a

Fig. 9

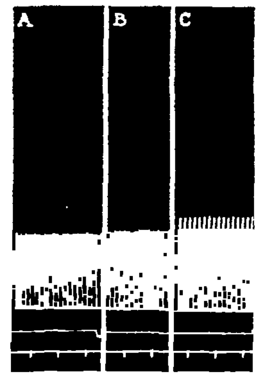

Fig. 10

Fig. 9. Effect of quinine on curarized muscle. Quadriceps; stimulated directly by multivibrator circuit after response to nerve stimulation had been abolished by curare. Lower signal: time in 5-sec. intervals; different spacing indicates different speed of drum. In each section the response to single stimuli at 5-sec. intervals is given just before the responses to tetanic stimulation. The upper signal indicates beginning and end of tetanic stimulations which were given at 2-minute intervals; the order of tetanic stimulations was 30, 72 and 125 per second in eacn instance. A, before quinine. B, 10 minutes after A; 10 mgm. of quinine hydrochloride had been given 9 minutes before and 15 mgm. 1 minute before. C, 8 minutes after B; 15 mgm. of quinine hydrochloride 7 minutes previously and 20 mgm. 0.5 minute previously. D, 20 minutes after C; 20 mgm. of quinine hydrochloride had been given 19 and 13 minutes previously and 30 mgm. had been given 7 minutes previously. At first signal 30 mgm. of quinine were injected.

Fig. 10. Potentiation of response to single maximal indirect stimuli by curare. Quadriceps; femoral nerve stimulated by multivibrator circuit as indicated. Time: 30-sec. intervals. A, before curare. B, 3.5 minutes after 0.005 cc. of curare injected intravenously. C, 7.5 minutes after B and 7 minutes after 0.01 cc. of curare.

period of rest is practically always prolonged, at times quite markedly. The second and succeeding contractions show only a slight prolongation which may decrease with continued stimulation. After a period of rest of 2 to 3 minutes a prolonged first contraction can again be obtained. Rosenblueth and Luco (1937) state that such prolonged contraction-remainders appear electrically as marked increases in the amplitude and frequency of the spike potentials in the spontaneous background. Injections of quinine inhibit the occurrence of the prolonged first contraction and may shorten the duration of succeeding contractions (fig. 11).

The denervated muscle shows the same sequence of responses to varying rates of stimulation as does the curarized muscle. With smaller doses of quinine the response to slow rates of stimulation is increased and the response to rapid rates of stimulation decreased. With successive doses of quinine the depression occurs at lower rates of stimulation and finally occurs at all rates. Again the doses of quinine required are much larger than in the indirectly stimulated muscles and the depression obtained is never so great. A plus-minus-plus response was not seen in either curarized or denervated muscles.

4. Effect on the response to acetylcholine. Quinine decreases both contraction and contracture resulting from intra-arterially injected acetylcholine.

TABLE 1

NUMBER OF ANIMALS		DURATION OF EXPERIMENT	DOSE OF QUININE HYDROCHLORIDE (GIVEN SUBCUTANEOUSLY TWICE DAILY)	PERCENTAGE OF ATROPHY OF DENERVATED MUSCLE AS COMPARED TO NORMAL	
Controls	Quinine treated			Controls	Quinine treated
		days	*mgm. per 100 grams*		
5	5	7	8	32.4	33.2
10	8	10	4	36.6	35.5
3	4	10	8	36.7	40.9
4	6	14	8	45.2	46.1

Discussion. The marked increase in response to single maximal indirect stimuli which eserine produces has been attributed to its anticholinesterase activity. It is therefore interesting to note that quinine also has a strong anticholinesterase activity (Matthes, 1930; Vahlquist, 1935). Although this activity is much less than that of eserine, the larger doses of quinine used may equalize the actual effects obtained in the animal. In addition to the increase in response to single maximal stimuli, certain other actions of quinine remind one of eserine: e.g., the plus-minus-plus response which is seen at moderate rates of indirect stimulation; the more complete tetanus obtained at slower rates of stimulation; the depression of response at high rates of stimulation. However, the absence of any evidence of repetitiveness in the action potentials and the occurrence of the quinine effect in curarized and denervated muscles show that the important influence of quinine is on the muscle and that the anticholinesterase activity probably plays little part in the observed phenomena.

The indirectly and directly stimulated muscles show the same sequence of events to varying rates of stimulation; that is, increase in response at slow rates and depression at rapid rates with depression occurring at progressively slower rates of stimulation as the dose of quinine is increased.

The increased response following both direct and indirect stimulation is undoubtedly due to an action of quinine directly on the muscle. This is indicated by the persistence of the increased response in the curarized and denervated muscles and by the absence of any evidence of repetitiveness in the action currents. The occurrence of the depression to indirect stimulation, however, is apparently not due to the changes in the muscle, for when the response to indirect stimulation is practically gone the muscle still responds very well to slow rates of direct stimulation and fairly well to moderate rates. The depression in the response of the indirectly stimulated muscle appear to be due to an effect of quinine on neuromuscular transmission. (The possibility of changes in nerve conduction have been mentioned, p. 231). Neuromuscular transmission and muscle response are apparently affected in a similar manner, but neuromuscular transmission is more sensitive to the action of quinine. The muscle and neuromuscular transmission behave as though, with quinine, each contraction is followed by a period of slow recovery during

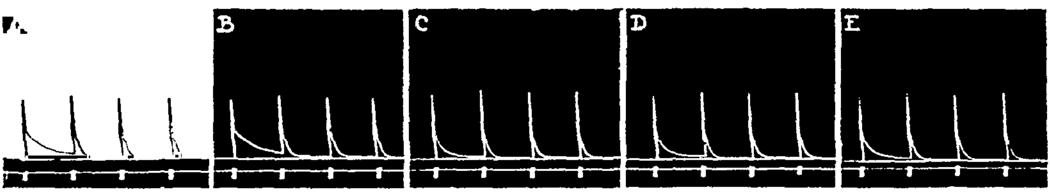

Fig. 11. Effect of quinine on the prolonged first contraction of denervated muscle. Quadriceps, denervated 14 days. Adrenals ligated. Condenser shocks. Time: 5-sec. intervals. Series of 4 contractions were taken at 3-minute intervals. A and B before quinine. C, 4 minutes after 15 mgm. of quinine hydrochloride. D, 10 minutes after quinine. E, 22 minutes after quinine.

which another stimulus produces a decreased response; in the muscle, in addition, this phase is followed by a supranormal period during which the response is increased. The duration of the subnormal phase increases with increasing doses of quinine. It is interesting to note that Briscoe (1935) has shown that curare also, in small doses, will inhibit the response to rapid (150 to 160) rates of stimulation at a time when the response to slower (20 to 35) rates is only slightly affected.

Eserine and tetani are much less effective in overcoming the neuromuscular transmission block produced by quinine than that produced by curare. It appears probable that curare may have the quinine-like action of increasing the response of a muscle as a result of a direct action on the muscle. With very small doses of curare it is possible to obtain an increase in response of a muscle to slow (1 every 5 sec.) indirect stimulation. This was noted by Rossbach in 1876 and is shown in figure 10. In view of the marked effect of curare in inhibiting repetitive responses arising at the neuromuscular junction, it seems likely that this increased response is due to a direct action on the muscle.

In table 2 is shown the striking ability of quinine to antagonize the influence of eserine on skeletal muscle. In some respects it is more effective than curare, as in the actions on denervated muscle. The ability of eserine to antagonize quinine is, however, much less than its ability to antagonize curare, and in this respect the quinine-eserine antagonism is not as complete as the curare-eserine antagonism.

The negative results obtained in testing the effect of quinine on the atrophy of denervated muscle do not disprove the hypothesis of fibrillary activity as a cause of the denervation atrophy. The doses of quinine given were in some instances large, and they might be expected to have an effect on the fibrillary activity, but how marked the effect and how long it lasted

TABLE 2

ESERINE	QUININE
Increases the response to single maximal nerve stimuli	Inhibits or prevents the increase to single maximal nerve stimuli produced by eserine
Depresses the response to indirect tetanic stimulation and favors the occurrence of Wedensky inhibition at low frequencies of stimulation	Counteracts the depression to tetanic stimulation and the Wedensky inhibition at low frequencies produced by eserine
Has a decurarizing action	Has a curare-like action and inhibits the decurarizing action of eserine
Increases the post-tetanic potentiation of single maximal stimuli	Decreases the post-tetanic potentiation
Produces spontaneous twitchings of peripheral origin	Inhibits the spontaneous twitchings produced by eserine (Weiss, 1926)
Increases the spontaneous fibrillation of denervated muscle (Rosenblueth and Luco, 1937)	Decreases the spontaneous fibrillation of denervated muscle
Increases the response of denervated muscle to acetylcholine	Decreases the response of denervated muscle to acetylcholine

was not determined. It was felt, however, that the experimental method was sensitive enough to have shown some inhibition of the atrophy if the atrophy were a result of the fibrillation. If the failure to obtain inhibition of atrophy was due to inadequate dosage of quinine, the possibility of therapeutically inhibiting denervation atrophy in man by the use of quinine seems out of question. In a recent preliminary report Solandt and Magladery (1940) state that they were unable to influence the atrophy of denervated muscles of albino rats with quinidine.

Of the many ways in which the denervated muscle resembles the myotonic muscle the prolongation of the first contraction after a period of rest is striking and significant. The characteristics of this prolonged contraction, and the ability of quinine and several other substances (po-

tassium chloride, adrenaline and calcium chloride) to inhibit it, form the basis of another report in which the marked similarities between the denervated and myotonic muscles are discussed in full.

SUMMARY

Quinine in small doses increases the response of a muscle to slow rates of direct or indirect stimulation while depressing the response to high rates of stimulation. With increasing doses of quinine the rate of stimulation required to show depression decreases, and finally depression is evident at all rates of stimulation (figs. 1, 3, 4, 9). Larger doses of quinine are required to produce the depression in directly stimulated muscle than in indirectly stimulated muscles; the probable explanation for this is discussed (p. 237).

Quinine antagonizes the actions of eserine on skeletal muscles as effectively as, and in some instances more effectively than, curare (table 2). Eserine is, however, not as effective in antagonizing the actions of quinine as it is in antagonizing the actions of curare.

Quinine inhibits the fibrillation of denervated muscle but an attempt to inhibit the atrophy of denervation in the gastrocnemius-soleus muscle of the white rat was unsuccessful (table 1).

The prolonged first response to electrical stimulation which a denervated muscle shows and which has many properties in common with myotonic contractions is inhibited by quinine (fig. 11).

I am indebted to Dr. W. B. Cannon and Dr. A. Rosenblueth for their interest and many helpful suggestions.

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THE RELAXATION EFFECT OF ACETYLCHOLINE ON THE OVIDUCT OF THE RABBIT IN RELATION TO HORMONAL STATUS

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Although numerous studies have been made on the effects of adrenaline on the tube and uterus, there are few investigations on the action of acetylcholine on the Fallopian tube. Li, (1935) in his experiments on the isolated oviduct of the monkey found inconstant and variable extent of contractions with acetylcholine perfusions. He found no definite relationship between the adrenaline or acetylcholine response of the Fallopian tube and the menstrual cycle.

In a previous communication (Davids and Bender, 1940) it was reported that intravenous injections of epinephrine produce contractions of the intact oviduct of the rabbit. These contractions are more pronounced during estrus or after the administration of estrogenic substances and are diminished on treatment with testosterone propionate.

At present we call attention to the effects of acetylcholine on the intact oviducts of rabbits in different hormonal states. In many instances comparative studies were made between the two "reciprocally active" substances, namely, adrenaline and acetylcholine.

Method. Mature female rabbits, weighing on the average of 3.2 kgm. were used in all experiments. The tubal contractions were recorded by the kymographic technique as described by Rubin (1927), Wimpfheimer and Ferested (1939), and Davids and Bender (1940).

In several rabbits simultaneous records of uterine contractions were made. The technique was that devised by Reynolds, (1930). A tubular balloon was inserted into the uterine cavity and connected to a bellows. Water was used to transmit the fluctuations in the balloon caused by uterine contractions.

The acetylcholine solutions were prepared from the powder (Merck & Co.) dissolved in normal saline. A constant volume of 1 cc. and constant speed of two seconds' injection were used in all trials. The eserine salicylate

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used to potentiate, and the atropine sulfate used to block the actions of acetylcholine, were given intravenously. In several rabbits bilateral adrenalectomy was performed through the abdominal surgical wound. In many instances the effects of acetylcholine on the tubal contraction during anestrus were compared with those observed during estrus or following the injections of estrogenic or androgenic hormones. Many side effects of acetylcholine such as lacrimation, salivation and defecation were noted and used as an indication of the degree of acetylcholine intoxication.

RESULTS. I. *Anestrus.* The oviducts of twenty anestrus rabbits were insufflated and tested with intravenous injection of acetylcholine.

In eight of these rabbits intravenous injection of 10 gamma of acetylcholine produced a frank relaxation of the tube (fig. 1). With previous eserinization, 0.6 mgm. injected intravenously, the relaxation was somewhat more pronounced.[3] Eserine alone produced no visible alteration in the tubal contractions. Atropine sulfate 1 mgm., injected intravenously one to ten minutes before the acetylcholine, blocked this relaxation. In

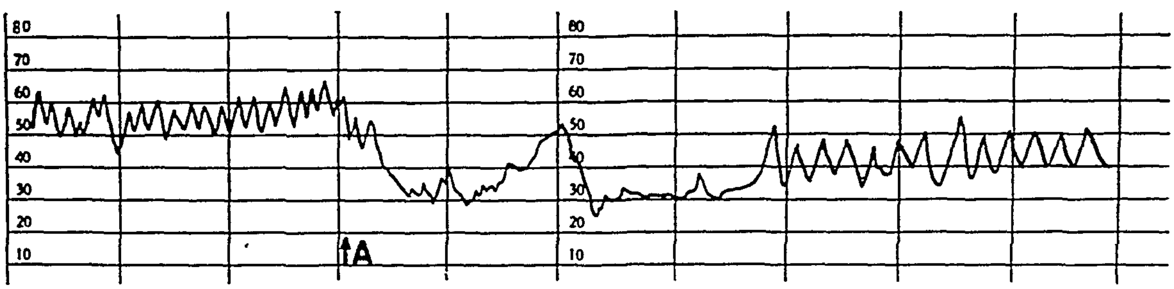

Fig. 1. Tubal contractions of a rabbit in estrus. Weight 3.5 kgm. At *A*, acetylcholine 20 gamma was injected intravenously. Abscissa, time in minutes.

two instances the inhibitory effect of acetylcholine could be demonstrated in the oviduct severed from the uterus, the insufflation of the CO_2 being made through the fimbriated end. The acetylcholine effect usually appeared from twelve to twenty seconds after the administration of the drug.

In four rabbits the relaxation effect of acetylcholine could be demonstrated only after a persistent contraction of the oviduct obtained by intramuscular injection of adrenaline (fig. 2). Adrenaline 0.1 to 0.3 mgm. per kgm. of body weight when injected intramuscularly produced a sustained contraction or spasm in the oviduct which lasted at times as long as fifteen minutes. This reaction to intramuscular adrenaline simulated that observed after intravenous injections. The response to intramuscular injection of adrenaline was increased during estrus or following administration of estrogenic substances and decreased or prevented after the injection of testosterone propionate.

[3] All tests with acetylcholine were made in uneserinized rabbits unless otherwise stated.

The relaxation effect of acetylcholine could also be demonstrated by intramuscular injections. Potentiation with eserine was difficult to elicit. Atropine sulfate always annulled the acetylcholine responses. The duration of the blocking action, however, depended on the amount of atropine given. One milligram of atropine sulfate given intravenously prevented the acetylcholine effect for ten to fifteen minutes.

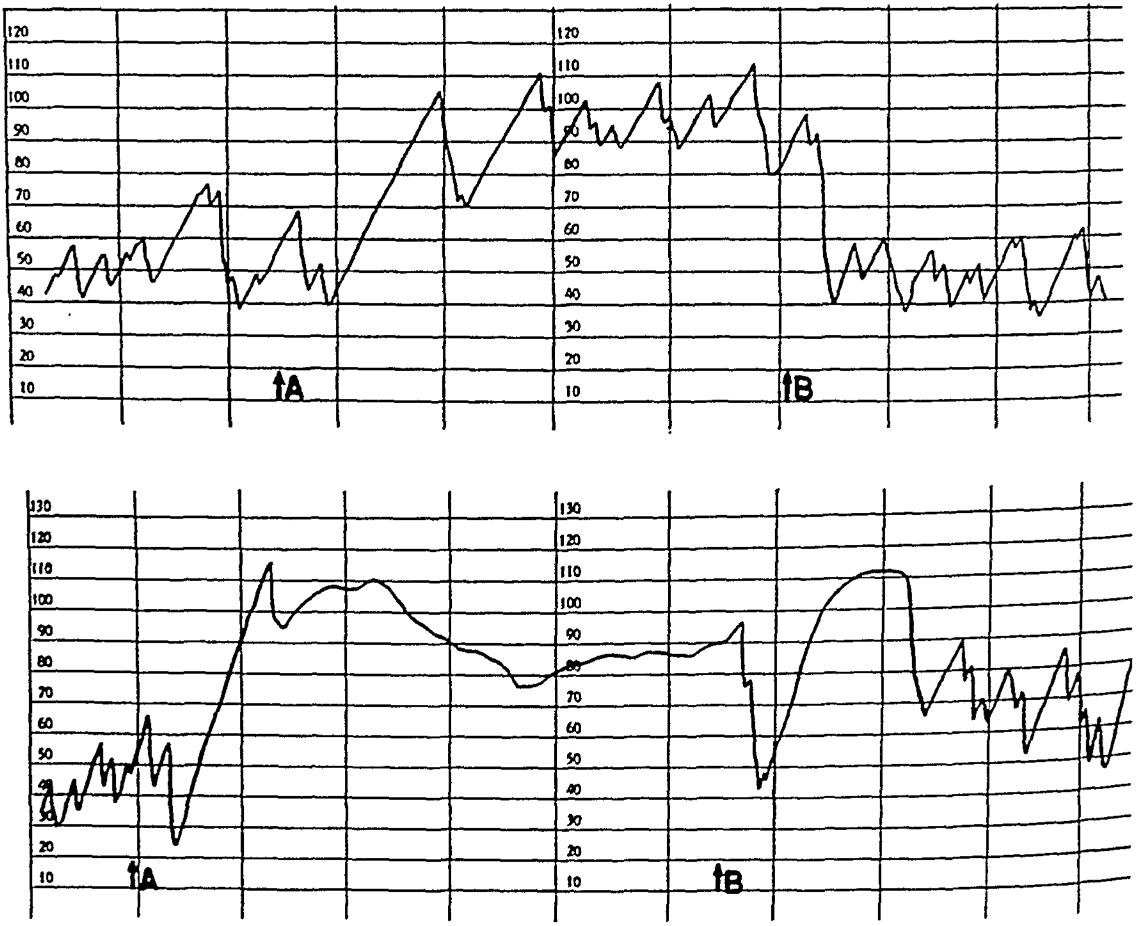

Fig. 2. (a) Upper: tubal contractions of rabbits in anestrus. Weight 3.3 kgm. At *A*, adrenaline 0.7 mgm. was injected intramuscularly. At *B*, acetylcholine 20 gamma was injected intravenously.

(b) Lower, weight 3.2 kgm. At *A*, adrenaline 0.8 mgm. was injected intramuscularly. Note the tubal spasm characterized by lack of fluctuations in the curve. At *B*, acetylcholine 10 gamma was injected intravenously.

In four rabbits acetylcholine in 10 or 20 gamma doses produced a contraction of the tube of the same magnitude as that obtained with adrenaline. This contraction sometimes appeared thirty seconds after the injection of the drug. The contraction was abolished by atropinization but not by bilateral adrenalectomy in one experiment. Two of these

rabbits exhibited both contraction and relaxation of the tube with acetylcholine, "diphasic effects"; the other two rabbits showed no relaxation effects.

In four other rabbits acetylcholine produced neither relaxation nor contraction of the tube. Eserinization or increasing the dosage of acetylcholine was also without effect.

2. *Estrus.* Eight rabbits were tested during estrus. In six of these estrus rabbits acetylcholine 10 gamma per rabbit, when injected intravenously produced a precipitous drop in the level of tubal contraction curve. Eserine salicylate slightly enhanced this relaxation while atropine (1 mgm.) always blocked it. A synchronous record of uterine contraction by

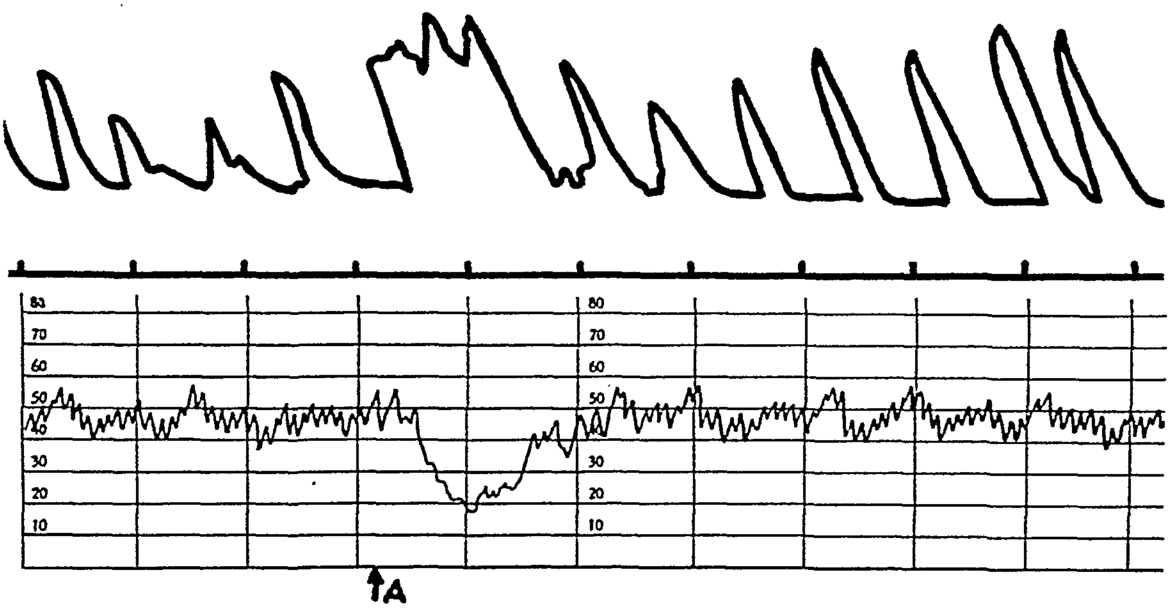

Fig. 3. Simultaneous record of uterine (upper tracing) and tubal (lower tracing) contractions. Rabbit in estrus. Weight 3.3 kgm. At *A*, acetylcholine 10 gamma was injected intravenously. Note contraction spasm in uterine and relaxation in tubal curves. Abscissa, time in minutes.

the Reynolds balloon method was made in four rabbits. Characteristic spontaneous uterine contractions were noted. The frequency of the uterine contractions was less than the tubal contractions which were simultaneously recorded. Intravenous injections of acetylcholine 10 gamma per rabbit, in these preparations, resulted in a contraction of the uterus and simultaneous relaxation of the tube (fig. 3). These results indicate that in general acetylcholine has a motor effect on the uterus and inhibitory action on the oviduct.

In two of the estrus rabbits acetylcholine produced neither motor nor inhibitory effect on the tubal contraction curve.

3. *Estradiol benzoate.* Four anestrus rabbits were injected intramus-

cularly with divided doses of estradiol benzoate (Roche Organon), the total doses varying from two to four thousand international units. Two to three days later these rabbits were tested with acetylcholine.

In three of these, intravenous injection of acetylcholine (10 gamma) produced a relaxation of the tube and in one a contraction. The inhibitory effect of acetylcholine was particularly conspicuous when the oviduct was in spasm due to intramuscularly injected adrenaline.

4. *Testosterone propionate.* Eleven anestrus and estrus rabbits were injected intramuscularly with testosterone propionate (total doses 100 to 200 mgm.) over a two week interval.

In only one of these rabbits did intravenous injection of acetylcholine produce a relaxation of the tube and this was not conspicuous. In four rabbits the intravenously injected acetylcholine (10 gamma per rabbit) produced a transient contraction of the tube, and in six rabbits acetylcholine was without effect. The contraction produced by acetylcholine was always blocked by atropine.

Adrenaline injected intramuscularly in the same doses which produced a prolonged and almost spastic contraction in the anestrus, estrus or estradiol benzoate treated rabbits, was conspicuously without effect in the rabbits treated with testosterone propionate. Only in two rabbits so treated did intramuscularly injected adrenaline (0.8 to 1.2 mgm.) produce a slight and sustained contraction of the tube. It seemed that testosterone propionate interfered with the actions of the injected adrenaline and acetylcholine on the oviduct.

5. *Castration.* Three rabbits were surgically castrated and tested with acetylcholine four weeks hence.

In all three rabbits castration did not alter the relaxation effect of intravenously injected acetylcholine. The degree of relaxation was not as great as in the anestrus and estrus rabbits before castration. Eserine and atropine exhibited the same properties as recorded previously.

6. *Injection with pregnant urine.* Three anestrus rabbits were injected intravenously with urine of pregnant women. Intravenous injections of acetylcholine in these rabbits three and four days later produced a relaxation effect in two of the rabbits.

Discussion. The foregoing experimental data reveal several interesting facts. The first is that acetylcholine injected intravenously in small doses into the uneserinized rabbit has an inhibitory influence on the muscle tone of the oviduct and excitatory action on the uterus. The relaxation property of acetylcholine is directly antagonistic to that of adrenaline on the same organ. These reciprocal chemical effects confirm the assumption that the sympathetic nerves exert a stimulating or contractile while the parasympathetic nerves exert an inhibitory influence on the Fallopian

tube. Incidentally the relaxation effect of acetylcholine on the tube is another example of inhibitory action of this drug, the others being dilatation of blood vessels, relaxation of ileocolic sphincter and inhibition of heart action.

Second is the influence of sex hormones on the inhibitory property of acetylcholine. It should be noted that during estrus and following the intramuscular injection of estrogens most of the effects produced by acetylcholine are inhibitory, whereas following treatment with testosterone propionate the relaxation influence of acetylcholine on the tube is negligible. From these and previous observations (Davids and Bender, 1940; Rubin and Davids, 1940) it would appear that testosterone propionate suppresses while estrogens potentiate the general activity and reactivity of the oviduct of the rabbit to adrenaline and acetylcholine.

The third is the less common contractile effect of acetylcholine on the oviduct. This motor effect may be explained by the well known observations that acetylcholine stimulates the adrenals and autonomic ganglia causing a secretion of adrenaline from adrenal gland and "sympathin" from sympathetic nerve endings throughout the body; the liberated adrenergic hormones, more stable than the acetylcholine injected into the blood stream, in turn act on the oviduct and thus produce a contraction. In some instances the relaxation effect of the injected acetylcholine and contractile effect of the reflexly liberated adrenergic substances neutralize each other, and no reaction is obtained. Still in other cases both inhibitory and motor effects are obtainable, "diphasic effects". Similar "diphasic effects" of acetylcholine have been found in experiments on the denervated iris of the cat by Bender and Weinstein (1940). As in case of the denervated iris all of the acetylcholine effects on the oviduct were abolished by atropinization. The fact that adrenalectomy did not annul the motor effect of acetylcholine on the tube suggests that a substance reflexly liberated at sympathetic nerve endings contracts the oviduct.

An alternate explanation for the double reactions of acetylcholine is that the smooth muscle cells of the oviduct may contain hypothetical chemical "receptive substances" (Langley, 1905) which unite with acetylcholine. When the injected acetylcholine combines with the excitatory factor, there results a contraction. When it unites with the inhibitory factor, there results relaxation.

Any chemical change in the hypothetical receptive substance such as brought about by treatment with sex hormones may alter the response to acetylcholine as observed in our experiments. Thus a contraction instead of relaxation of the tube may occur after treatment with testosterone propionate. The same theory may explain the reversal of uterine response to adrenaline during pregnancy (Kennard, 1937).

SUMMARY

1. The effects of acetylcholine on the intact oviduct of rabbit were studied by the tubal insufflation method.

2. Acetylcholine has in general an inhibitory effect on the oviduct which is directly antagonistic to the motor action of adrenaline on the same organ.

3. While acetylcholine relaxes the tube it simultaneously produces a contraction of the uterus in the estrus rabbit.

4. The hormonal state affects the tubal response to intravenously injected acetylcholine.

5. In some rabbits acetylcholine produces a contraction in the tube; in others both relaxation and contraction, "diphasic effects", are found.

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EXPERIMENTS ON THE VASCULAR SUPPLY OF THE RABBIT'S HYPOPHYSIS[1]

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Recent increases in knowledge of the functional relations between hypothalamus and hypophysis have taken place largely as a result of surgical interruption of the connections between these parts. This is especially true of the studies on the rôle of the neurohypophysis in the control of water balance (1). The technique has also been used in determining the importance of hypothalamico-hypophysial connections in the discharge of certain hormones from the anterior lobe (2, 3). The functional disturbances caused by these operations have been attributed by almost all workers to severance of neural connections between the hypothalamus and infundibular process or the anterior lobe. Yet the possibility exists that as a result of section of the hypophysial stalk or of the placing of electrolytic lesions in its neighborhood vascular injury may take place. Thus the functioning of cells in the hypophysis might be affected seriously. This was, in fact, the basis for the belief of Mahoney and Sheehan (4) that nervous connections between hypothalamus and pars nervosa in the dog may not be important in the control of water intake and output. Others have considered the possible significance of vascular disturbances in the region of the stalk produced by operative injury but regarded them of no great importance (5). The experiments described in this paper were carried out in order to determine the effect on the hypophysial vascular bed of depriving the gland of one or more sources of arterial blood.

In the rabbit Wislocki and King (6) have shown that arterial blood reaches the hypophysis through a large number of arteries of small caliber derived from the circle of Willis. These afferent vessels enter the gland either directly or after passing along the stalk. Some of them bear blood which has already passed through a capillary bed in the stalk or median eminence. Several of the arteries are large enough to be seen with the unaided eye and can be ligated or cut with ease. There are two or three such vessels which arise on each side from the circle of Willis near the point where it is joined by the internal carotid artery. These pass medially

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toward the stalk. More caudally there are two other vessels, one on each side, which leave the arterial circle near the basilar artery and pass forward toward the hypophysis. In addition to these macroscopic vessels derived from the circle of Willis a pair of arteries arise from the internal carotids and enter the posterior lobe of the hypophysis. In summary, the chief sources of arterial blood to the hypophysis are the following: 1, vessels which pass in or around the stalk toward the pituitary gland; 2, lateral twigs which pass from the circle of Willis toward the hypophysis independently of the stalk; 3, posterior twigs arising from the caudal part of the circle of Willis to enter the hypophysis directly; 4, arteries of the posterior lobe derived directly from the internal carotid arteries.

PROCEDURE. The following procedures were used to test the importance of each of the above sources of arterial blood in the maintenance of the capillary bed of the hypophysis.

Adult rabbits were killed with illuminating gas. Immediately thereafter the hypophysis was deprived of one or more sources of arterial blood by cutting the vessels concerned or by occluding them with silk ligatures or silver clips. This was followed by injection of India ink (diluted with an equal volume of water) through the aorta at a pressure of approximately 120 mm. of Hg. When the injection was judged to be complete the hypophysis was dissected out carefully, fixed in formalin, embedded in celloidin and sections 100 μ thick were cut serially. The sections were studied unstained and the injected areas mapped accurately. Maps of several glands deprived of the same arterial supply were superimposed and the composite was compared with the blood vessel pattern of three normal glands injected in the same way but with no interference to their blood supply. Particular attention was paid to capillary anastomoses between the different lobes of the hypophysis.

The vascular operations preceding the injection of India ink were carried out as follows:

1. The stalk alone was cut in seven rabbits. Arterial blood could reach the hypophysis by way of the lateral and posterior twigs from the circle of Willis and from the arteries of the posterior lobe which have their origin in the internal carotids.

2. The lateral twigs from the circle of Willis and the adjacent internal carotid arteries were interrupted in six rabbits. Blood could then reach the hypophysis through the stalk vessels, through the posterior twigs from the circle of Willis (by way of the basilar artery) and through the posterior lobe arteries.

3. In three rabbits the stalk and lateral twigs were cut. After this operation blood could still reach the hypophysis through the postérior twigs and the posterior lobe arteries.

4. All vascular connections between the circle of Willis and the hypoph-

ysis were cut in three rabbits. In order to make sure that the stalk and blood vessels had been completely severed the brain was raised from the diaphragma sellae turcicae. Under these circumstances blood could reach the hypophysis only by way of the posterior lobe arteries from the internal carotids.

5. The basilar and both common carotid arteries were ligated and sectioned in two rabbits. Theoretically no blood should reach the hypophysis following this procedure.

Results. Excellent capillary injections that were almost complete were obtained in two of the three normal glands. The anterior lobe of the third was poorly injected in the region of its anterior inferior surface. In all cases the capillary bed of all parts of the hypophysis was quite continuous. Capillaries pass between infundibular process and the pars intermedia, between the latter and the anterior lobe around the edges of the residual lumen of Rathke's pouch, and between the infundibular stem and the pars distalis. Of the experimentally treated animals, the only other instance of a similarly injected hypophysis was one whose lateral twigs had been cut. In all other cases (except the two of group 5 in which the entire arterial circulation to the hypophysis was blocked) the capillaries were injected incompletely. In order to evaluate the irregularities in the extent to which vessels might be expected to be injected, diagrams of the injected region of the gland in different animals of each group were superimposed. The resulting composite picture was believed to represent more accurately the true extent of the entire capillary bed in the hypophysis following each type of opertaion. The composite capillary bed derived in this way is described below.

1. *Stalk alone cut.* The capillaries were injected as completely as in normal glands. A small number of capillary anastomoses between pars intermedia and the anterior lobe, and between the latter and the infundibular stem could be seen clearly in one specimen in which the only capillaries of the anterior lobe injected lay adjacent to the pars intermedia and the stalk and were continuous with their vessels. In this preparation they extended for a short distance only into the anterior lobe.

2. *Lateral twigs alone cut.* The capillaries were as completely injected as in normal glands.

3. *Stalk and lateral twigs cut.* The capillaries were fully injected as in the normal glands.

4. *Stalk, lateral twigs, posterior twigs and other vascular connections with circle of Willis cut.* The posterior lobe vessels were fully injected and they had the same appearance as in normal glands. Capillaries from pars intermedia and infundibular stem passed for a short distance into the pars anterior. They spread out from around the edge of the residual lumen of Rathke's pouch so as to cover its whole anterior surface. In two glands

one or more large veins from the anterior lobe were injected because of the back pressure of the injection fluid in the venous system; the capillaries in the surrounding region contained ink particles.

5. *Basilar and common carotid arteries cut.* No capillaries in the hypophysis were injected.

DISCUSSION AND CONCLUSIONS. One of the most prominent aspects of the observations described above is that a free anastomosis exists between the capillary beds of different, perhaps widely separate vascular regions of the pars anterior. For example, the integrity of only two of the large number of arteries normally present (such as posterior twigs from the circle of Willis) may be sufficient to assure the access of India ink to the capillaries of the whole lobe.

Another conclusion substantiated by these experiments is that injections of the various parts of the hypophysis can be obtained after destruction of the arterial and portal circulation in or around the stalk. In experiments in which the stalk was cut (even when the lateral twigs also were severed) the anterior lobe and the pars intermedia appeared quite well injected. The infundibular process and the pars intermedia were thoroughly injected even when the whole arterial supply of the hypophysis was interrupted except for those branches passing to the posterior lobe from the internal carotid arteries. To the limited extent to which this type of evidence is applicable, the stalk circulation does not appear to be essential to the completeness of hypophysial circulation.

One is impressed by the limited number of capillary anastomoses between pars intermedia and pars anterior. This has been demonstrated experimentally by Morato (7). The pars intermedia, so far as its circulation pattern is concerned, appears to be more closely related to the pars nervosa. This may be related to the fact that the surface between these two parts of the hypophysis is, of course, greater than that between pars intermedia and anterior lobe and offers a richer opportunity for capillary anastomoses.

Further implications are limited because the method of vascular injection is an indirect one. It is quite possible, for example, that the existence of a potential capillary circulation may be demonstrated by India ink injection for a region whose major blood supply is occluded and in which the rate of blood flow may be reduced to such a degree as to be virtually equivalent to complete stasis. On the other hand, the potential circulation may become a reality through reorganization of the vascular bed. The number of variables is too great to be controlled and clarified merely by the technique of India ink injections of acute preparations. Chronic experiments should throw more light on the situation. It is pertinent to recall that in some stalk-cut rabbits which survived a long time after operation Brooks (2) found that a small central scar developed in the anterior lobe. In a number of cases one or more of the arterial twigs

which run with or along the stalk were cut inadvertently as the stalk was being sectioned. Such animals sometimes showed signs of anterior lobe deficiency. In these cases, large areas of degeneration in the pars distalis were found at autopsy.

In spite of the limitations of the method employed it seems proper to conclude that stalk section alone should not interfere with the blood supply of the infundibular process in a surviving rabbit. Stalk section may possibly modify the circulation in the anterior lobe. If there are any ill effects of such an operation they may be minimized in a surviving rabbit by the free capillary anastomoses which exist within the gland. These conclusions have a clear bearing on the interpretation of the disturbances of water exchange and of certain reproductive mechanisms that follow stalk section. Since the effects of this operation on the circulation within the hypophysis are relatively unimportant, the abnormalities of function caused by stalk section must be related instead to interruption of the nerve pathways.

SUMMARY

In a series of acute experiments the various components of the vascular supply to the hypophysis of the rabbit were occluded singly or in various combinations. To determine the effects of these procedures the capillary bed of the gland was injected with India ink.

It was found that a free anastomosis exists between the capillary beds of different regions of the pars anterior. When only a portion of the arterial supply is left intact the capillaries of the whole lobe are perfused. Transection of the hypophysial stalk does not impair the completeness of the injection. Capillary anastomoses between this lobe and the pars intermedia are few in number. The pars nervosa and pars intermedia can be perfused completely by way of the posterior lobe arteries which run to the caudal aspect of the gland from the internal carotids.

Since the effect of stalk transection on the circulation within the hypophysis is relatively unimportant, the abnormalities of function caused by this operation must be due to interruption of nerve pathways.

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THE AVAILABILITY OF dl-THREONINE AND dl-ALLOTHREONINE FOR THE FORMATION OF CARBOHYDRATE[1]

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It was first demonstrated by McCoy, Meyer, and Rose (1) that an α-amino-β-hydroxy-n-butyric acid was a naturally occurring, essential amino acid. It was later demonstrated that the d(−) threonine, as it was named, was the naturally occurring form (2) and the only form essential for growth of the rat (3). Little is known as to the metabolic pathways followed by this amino acid or its isomers, though Knoop (4) has developed a theory concerning the metabolism of aliphatic β-hydroxy-amino acids. Snyder and Corley (5) on injecting dl-allothreonine concluded that it was not significantly deaminized.

The purpose of the present study was to determine whether or not dl-threonine or dl-allothreonine is available for the formation of carbohydrate in the rat.

EXPERIMENTAL. Two procedures were used to determine carbohydrate formation. The first, following the method used by Butts, Dunn and Hallman (7), was to determine the amount of liver glycogen produced after feeding the amino acids to rats previously maintained for 2 days on a low residue diet. Female rats 140 to 180 grams in weight were fasted for 24 hours and then fed a definite amount of the synthetic amino acid every two hours over a four or eight-hour period. The amount administered was always in excess of the absorbing capacity of the gastro-intestinal tract of the animal. The animals were allowed access to distilled water at all times and to filter paper throughout the 24 hour fasting period. At the conclusion of the experimental period sodium amytal was injected, the livers were removed, and glycogen determinations were made by the method of Good, Kramer and Somogyi (6). These results are given in table 1.

Since we found in a preliminary experiment that more glycogen was

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formed in eight hours than in four, the longer period of time was used in subsequent experiments. In these preliminary studies the rats were given 0.5 gram of amino acid per feeding. The almost universal diarrhea among the experimental animals and the distended, hyperemic conditions of the gastro-intestinal tract at the end of the experimental period indicated that the dose of amino acid was too large. Reduction of the dose to 0.3 gram per feeding avoided the diarrhea and resulted in an increased formation of glycogen. One group of four rats which was fed the larger dose of dl-threonine, but which did not develop diarrhea is included in the table (group C).

The data clearly demonstrate that both dl-threonine and dl-allothreonine form glycogen in the rat. The differences between the amount of glycogen formed from dl-threonine or dl-allothreonine are probably not significant. If one animal in group D (dl-allothreonine) which produced only 0.11 per cent of liver glycogen is excluded, since the result in that instance is obviously out of line with the other figures from the group, the average in this case becomes 0.64 per cent. This may be compared with 0.62 per cent of glycogen found for dl-threonine (group B). We doubt that any very significant comparison can be made between our figures and those of other investigators since there seems to be some difference between values for liver glycogen obtained with rats of different strains under similar experimental conditions and with similar previous nutrition. Furthermore, it would seem to us that direct comparisons of the glycogenic action of the various amino acids by this method would presuppose the same or very nearly the same rates of deamination. Snyder and Corley (5) found little, if any, deamination of dl-allothreonine in the dog. In preliminary studies, not to be reported in this paper, we have indications that dl-threonine is not deaminized as rapidly as glycine, alanine, or some of the other acids.

TABLE 1

Per cent of glycogen in rat livers

GROUP	NUMBER OF RATS IN GROUP	COMPOUND FED	RANGE	AVERAGE
A	12	Distilled water (control)	0.02-0.31	0.12
B	6	dl-Threonine, 0.3 gram every two hours for 8 hour period	0.34-0.83	0.62
C	4	dl-Threonine, 0.5 gram every two hours for 8 hour period	0.69-1.21	0.90
D	8	dl-Allothreonine, 0.3 gram every two hours for 8 hour period	0.11-0.92	0.58

The second procedure employed to determine carbohydrate formation was a method used by Butts, Dunn and Hallman (7). Male rats 125 to

185 grams in weight were fed 15.0 grams of sodium butyrate (as acetone) per square meter of body surface per day. The second, third and fourth days, the experimental animals were fed 15.4 grams of dl-threonine or dl-allothreonine per square meter per day and acetone body excretion was determined by the method of Van Slyke. Total urinary nitrogen excretion was determined by the Kjeldahl method. The results are given in table 2.

There was a significant reduction in acetone body excretion on feeding the amino acids with the sodium butyrate. This reduction in ketonuria indicates the potentialities of these amino acids for carbohydrate formation.

Though as previously mentioned, Snyder and Corley (5) concluded that dl-allothreonine was not significantly deaminized, when injected into the dog, this apparent contradiction with the present study is probably not as real as it seems. There appears to be a species difference in the metabolism of dl-allothreonine between the dog and the rat, though it might be pointed out that Snyder and Corley's figures do show slight increases in ammonia nitrogen or in urea and ammonia nitrogen excretion after injection of the dl-allothreonine. Our study obviously does not differentiate between the action of the optical isomers. It might be mentioned, however, that in a preliminary study with dogs there was no evidence, from a polariscopic examination of the urine, of a preferential utilization of either optical isomer of either the threonine or of the allothreonine.

TABLE 2

Acetone body excretion by male rats receiving 15.0 grams of butyric acid (calculated as acetone) per square meter per day together with dl-threonine or dl-allothreonine

NUMBER OF ANIMALS IN GROUP	AMINO ACID FED	AMOUNT OF ACETONE BODY EXCRETION MGM. PER 100 GRAMS OF RAT		
		1st day	2nd day	3rd day
3	None	122	161	165
4	dl-Threonine	71	85	74
4	dl-Allothreonine	77	84	84

SUMMARY

The feeding of either dl-threonine or dl-allothreonine resulted in definite increases in liver glycogen. Similarly, both substances decreased the ketonuria resulting from feeding butyric acid. This indicates that both dl-threonine and dl-allothreonine may be transformed to carbohydrate in the rat.

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THE EFFECT OF BILE ON THE QUANTITY AND DIGESTIVE ACTIVITY OF SMALL INTESTINAL JUICE[1]

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Although the control of the secretion of the small intestine has received little attention in the literature, certain facts regarding it have been quite well established. Extracts of the intestinal mucosa have been reported to contain a hormone, enterokrinin, which is capable of exciting the glands of both the innervated and the denervated small intestinal loop in the dog (5). Numerous observations indicate that mechanical stimulation as produced by distention serves as a potent stimulus to intestinal secretion (3, 4). The oral administration of such foods as eggs and milk, glucose solutions, and solutions of iron salts (7) and of peptone (6) have been found to result in an increased rate of secretion. On the other hand, observations of the effect of local application or presence of carbohydrate solutions, of tenth normal and twentieth normal hydrochloric acid and of bile salts failed to furnish evidence of any specific stimulating effect (7).

In the course of a study which demonstrated that propulsive motility of loops of the small intestine is increased following the local application of bile (1), increased amounts of intestinal juice were observed to flow from the fistula, apparently in response to the same stimulating influence (2). Five explanations for this increased flow of juice seemed possible. First, it might be produced as a result of the emptying action of increased propulsive movements on juice already present in the loop. Secondly, a reflex effect of a stimulating action of bile on visceral receptors would possibly explain the result. Thirdly, the liberation of a hormone comparable to Nassett's enterokrinin in response to a local action of bile might be postulated. Fourthly, although it was guarded against, the possibility remains that mechanical stimulation incident to the introduction of the test solutions was the cause of the increased secretion. Fifthly, the increased flow of juice might be caused by a local stimulating action of bile.

In order to obtain further evidence regarding a possible stimulating effect of bile on the glands of the small intestine, and to find whether the

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increased flow of juice was accompanied by an increase in enzyme output, the following experiments have been performed.

METHOD. One or two Thiry loops of about 8 to 10 cm. in length, and with distal ends to the exterior, were prepared in 7 dogs from the upper jejunum as near as convenient to the ligament of Treitz. Three groups of animals were used. In the first group only one Thiry loop was prepared; in the second, two innervated Thiry loops; and in the third, one innervated and one denervated loop.

After a training period of a few weeks during which the animal was taught to lie quietly throughout the period of a test, the following procedure was used.

The loop was thoroughly washed with isotonic saline buffered at pH 6.8 and at body temperature. The solution was introduced by means of a small catheter and syringe. Final content of the loop was carefully aspirated.

A 4 cc. portion of the solution to be tested was then introduced into the depths of the loop at 10 minute intervals until 6 applications had been made. Care was taken to produce as little mechanical stimulation as possible. The fluid draining from the fistula during the one hour collection period was collected in a small vessel held in such a position that no loss of fluid occurred except in rare instances, the results being discarded in the latter case. The constant presence of an attendant throughout the period of collection is essential.

At the end of the one hour collection period the loop was again washed with buffered isotonic saline, its final contents were aspirated, and the washings were pooled with the collection.

The enzymic activity of the fluid collected was determined for its ability to digest sucrose. Digestion mixtures were made up of one-fourth of one hour's collection from a loop, 25 cc. of 2 per cent sucrose buffered with phosphate buffer at pH 6.8, one-half cubic centimeter of toluene, one-half cubic centimeter of bile salt solution, and sufficient of the isotonic buffered saline to bring the volume to 50 cc. Two digestion mixtures were prepared for each specimen collected, one containing boiled and the other unboiled juice.

The glucose equivalent of the digests after 24 hours of incubation at 38°C. was determined on protein-free filtrates by means of the Shafer-Somogyi method. Data are given in terms of the difference in glucose equivalent of boiled and unboiled specimens; i.e., the net glucose equivalent.

Each experiment was preceded by a fast of 24 hours and included at least one control collection period of a duration of one hour and an experimental period of equal duration. During the control period saline solution buffered with phosphate at pH 6.8 was used and during the experimental

period either dogs' gall bladder bile or a 7½ per cent bile salt[2] solution similarly buffered to the same pH. All solutions were made approximately isotonic with blood as judged by determinations of the depression of the freezing point.

Additional controls with saline are described in 1, *d* of Results.

TABLE 1

Dogs having a single innervated Thiry loop

Dog number	Test hour	Solution used*	Volume change	Net reduction in mgm. glucose equiv.	Dog number	Test hour	Solution used*	Volume change	Net reduction in mgm. glucose equiv.
			cc.					*cc.*	
1	1st	Saline	0	497	3	1st	Saline	0	342
	2nd	Saline	−4	165		2nd	Bile	+7	510
3	1st	Saline	+1	384	4	1st	Saline	+1	540
	2nd	Saline	0	240		2nd	Bile	+9½	754
4	1st	Saline	0	434	4	1st	Saline	+½	472
	2nd	Saline	+1½	282		2nd	Bile	+7	787
1	1st	Saline	+1½	290	1	1st	Na taur.	+9	477
	2nd	Na taur.	+6	356		2nd	Saline	+3½	342
	3rd	Saline	+3½	181		3rd	Na taur.	+5½	461
2	1st	Saline	+½	398	2	1st	Na taur.	+1	358
	2nd	Na taur.	+2	484		2nd	Saline	−1½	65
	3rd	Saline	−½	201		3rd	Na taur.	0	293
4	1st	Saline	−½	673	3	1st	Na taur.	+7	683
	2nd	Na taur.	+10	827		2nd	Saline	+1½	227
	3rd	Saline	+1½	358		3rd	Na taur.	+7	564
4	1st	Saline	+1½	547	1	1st	Na glyco.	+6	423
	2nd	Na glyco.	+4	750		2nd	Saline	+1	168
5	1st	Saline	+2½	426		3rd	Na glyco.	+4	429
	2nd	Na glyco.	+4	606	2	1st	Na glyco.	+2½	181
5	1st	Saline	+1	411		2nd	Saline	+1	86
	2nd	Na glyco.	+6½	515		3rd	Na glyco.	+1½	146
					3	1st	Na glyco.	+6½	819
						2nd	Saline	+2½	233
						3rd	Na glyco.	+6½	535

* Na taur. = sodium taurocholate solution; Na glyco. = sodium glycocholate solution.

Results: 1. Five dogs with a single innervated Thiry loop. *a.* The effect of application of dogs' gall bladder bile. In 4 of 5 experiments on one dog and in all of the 3 experiments on a second dog marked increases in net glucose equivalent were found in the digests representing the period during which bile was used, as compared with the control period. The

[2] Pfanstiehl.

increases ranged from 22 per cent to 66 per cent. See data of table 1. Changes in volume during the control period ranged from 0 to +2 cc. and during the period of bile application from +4 cc. to +12 cc.

b. The effect of sodium taurocholate solution. In all of 18 experiments on 4 dogs there was a marked increase in net glucose equivalent in the digests representing the bile salt solution as compared with those of the control. These increases ranged from 21 per cent to 450 per cent above the corresponding controls. Volume changes for the controls ranged from $-1\frac{1}{2}$ cc. to +4 cc., and for the period during which the bile salt was applied from +1 cc. to +12 cc. Thirteen of 18 tests using the bile salt solution showed increases in volume of more than 5 cc. over that of the controls. Representative data are given in table 1.

c. The effect of sodium glycocholate solution. In 11 experiments on 4 dogs all except one showed an increase in net glucose equivalent above 37 per cent, the increase ranging up to 763 per cent. Volume changes for the controls ranged from −3 cc. to +5 cc. and for the bile salt solution from $+1\frac{1}{2}$ cc. to $+9\frac{1}{2}$ cc. See table 1 for representative data.

d. The effect of repetition of the control solution, buffered isotonic saline, during a second consecutive hour. In 9 of 14 experiments on 4 dogs there was less digestion during the second hour than during the first; while of the other 5 experiments, 2 showed no change and 3 showed slight increases of 14 per cent, 18 per cent and 14 per cent, respectively. Representative data are to be found in table 1. Volume changes ranged from 0 to +4 cc. during the first hour and from −4 cc. to +3 cc. during the second hour.

2. In 2 dogs with two innervated Thiry loops each, the control solution was used during two consecutive hours in loop I while in loop II the control solution was used during the first hour and bile or one of the bile salt solutions during the second hour. Any reflex effect of the experimental solutions should then be detectable in the response of loop I. In loop II the response was similar to that observed in other experiments with the same test solution. In 11 of 12 experiments on 2 dogs the increases in net glucose equivalent ranged from 12 per cent to 168 per cent, and one showed a decrease of 2.5 per cent. Nine of the 12 showed an increase of 25 per cent or over, and 6 an increase of 34 per cent or over. In the same experiments loop I behaved in response to the control solution as though it were independent of reflex effects from loop II. Thus in 10 of the 12 experiments there was a reduction of net glucose equivalent in the specimen of the second hour as compared to the first, while in only 2 was there a slight increase, in one case of 17 per cent and in the other of 18 per cent. Similarly the volume changes failed to indicate any effect of introduction of bile or bile salt solutions into one loop on the secretion of the other. Examples of these experiments are given in table 2.

3. In 2 dogs with loops that had been denervated by mesenteric stripping and application of phenol to the vessels, the response to sodium taurocholate solution was fully as great as in the case of normal dogs. Thus in 6 of 7 experiments on 2 dogs the increases in net glucose equivalent ranged from 42 per cent to 134 per cent and in the seventh 10 per cent.

TABLE 2

Dogs with two Thiry loops. Effect of introduction of test solution into one loop on juice from second loop

DOG NUMBER	LOOP NUMBER	TEST HOUR	SOLUTION USED	VOLUME CHANGE	NET REDUCTION IN MGM. GLUCOSE EQUIV.
				cc.	
4	I	1st	Saline	−2	362
		2nd	Saline	−2	247
	II	1st	Saline	+2	393
		2nd	Bile	+5	527
5	I	1st	Saline	0	255
		2nd	Saline	+1	114
	II	1st	Saline	+½	256
		2nd	Na taur.*	+8	688

* Sodium taurocholate solution.

TABLE 3

Dogs with denervated Thiry loops

DOG NUMBER	TEST HOUR	SOLUTION USED*	VOLUME CHANGE	NET REDUCTION IN MGM. GLUCOSE EQUIV.	DOG NUMBER	TEST HOUR	SOLUTION USED*	VOLUME CHANGE	NET REDUCTION IN MGM. GLUCOSE EQUIV.
			cc.					cc.	
6	1st	Saline	+1½	395	7	1st	Saline	−3	291
	2nd	Na taur.	+9½	925		2nd	Na glyco.	+3½	621
6	1st	Saline	−1	184	6	1st	Saline	+2	339
	2nd	Na taur.	+4½	369		2nd	Na glyco.	+4	540
7	1st	Saline	−2	149	7	1st	Saline	+2	625
	2nd	Na taur.	+4	289		2nd	Na taur.	+6	885

* Na taur. = sodium taurocholate solution; Na glyco. = sodium glycocholate solution.

Volume changes in the saline controls ranged from −3 cc. to +2 cc. and in those using bile salt solution from +2 cc. to +9½ cc. It would seem then that the effect of locally applied bile and bile salts on the volume and digestive activity of the juice collected from loops of the small intestine does not depend on their extrinsic nerve supply. Representative data are presented in table 3.

DISCUSSION. As indicated in the recent report of Wright et al. (9), there has been some question as to whether intestinal juice owes its disaccharide-splitting power to exo-enzymes secreted by gland cells or to endoenzymes present in suspended mucosal cells. Pierce et al. (8) found that centrifuged intestinal juice has a more feeble digestive action on sucrose and other substrates than does uncentrifuged juice. They attributed the difference to the presence in the latter of a suspension of amorphous material and a few mucosal cells, and suggested that the enzymes might be adsorbed by this material.

In the present work the collected juice was shaken with glass beads as suggested by Pierce et al. (8) before the sample to be incubated was withdrawn from the collecting vessel. Since the amount of mechanical stimulation incident to the introduction of the test solution and various control solutions was in all cases approximately equal, it would seem that the shedding of increased numbers of mucosal cells containing invertase would not account for the increases in digestive action found. While the possibility that local action of bile or bile salts might conceivably result in the shedding of mucosal cells must be considered, such an effect would in no sense detract from the importance of the action of bile in causing increase in volume and in disaccharide-splitting action of intestinal juice.

The fact that the increases in secretion were demonstrated in previously washed loops seems to rule out the possibility that increase in outflow of juice observed in previous experiments is caused by increased propulsive movements.

The possibility of a reflex mechanism which would account for the increase in secretion following application of bile is ruled out by the good responses obtained from denervated loops.

The failure to obtain an increase in secretion of a second Thiry loop while the first one was being stimulated by locally applied bile salt solution argues against a hormonal as well as a reflex activation of the glands in response to bile salt solution.

The fact that mechanical stimulation incident to the introduction of test solutions was equal in the case of control and experimental solutions would seem to rule out this mode of stimulation as a possible causative agent. It may still be claimed however that the increase in propulsive movements which follows application of bile salts possibly might result in mechanical stimulation of the glands. It is questionable however whether these movements are sufficiently intense to provoke such a stimulation.

A local stimulating action of bile salts on the glands of the small intestine readily explains the increased secretion observed in these experiments. The relative importance of this action in the normal physiology of the small intestine is not entirely apparent. It would appear that bile salts may well serve as a part of the normal mechanism for stimulation of the

glands of the small intestine and that through this means they aid in digestion. In the absence of bile salts the rate of secretion of the small intestinal glands may be expected to be considerably less than during their presence.

SUMMARY

Gall bladder bile of the dog was applied locally to the mucosa of Thiry loops of the jejunum of dogs, and the outflow of fluid from the loop was collected. During a given collection period when bile was applied, the specimen obtained in unit time was greater in volume and in its ability to digest sucrose than were similar specimens collected during application of isotonic saline buffered at pH 6.8. Local applications of both sodium glycocholate and sodium taurocholate in 7.5 per cent solution adjusted to isotonicity by addition of sodium chloride and buffered with phosphate at pH 6.8 produced results essentially the same as those obtained with bile.

Application of bile or bile salt solution to the mucosa of one of two innervated Thiry loops in a given dog failed to influence the secretion of the other loop.

Denervated Thiry loops responded fully as well as, but not better than, those whose innervation remained intact.

It is concluded that local application of bile to the mucosa of jejunal loops in the dog results in increased volume and digestive activity of the intestinal juice collected in unit time. In the light of the data presented a local stimulating action of bile salts on the glands of the jejunal mucosa best explains this result.

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VITAMIN E DEFICIENCY IN THE MOUSE[1]

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Proof of the existence of vitamin E is based primarily upon the characteristic impairment of reproduction observed in rats reared on highly purified diets deficient in this substance but adequate in all other respects. Evidence concerning the need of this vitamin for reproductive function in other vertebrates is either of circumstantial nature or is based upon the use of diets of natural foodstuffs subjected to chemical treatment (ferric chloride in ether) in an effort to destroy or inactivate the vitamin E present. Considerable evidence has been obtained in this laboratory (unpublished studies) indicating that the latter objective is not always attained by this procedure. Attempts to induce a state of vitamin E depletion in the mouse, the only other laboratory animal which readily consumes the concentrated and purified type of diet such as used for rats, appear to be limited to the early studies of Beard (1926). Since the diet used in the latter studies contained 20 per cent of hydrogenated cottonseed oil (Crisco), which has since been shown to constitute a very good source of vitamin E, it is evident that the sterility observed in mice of both sexes cannot be attributed to an inadequacy of this vitamin and must be explained on some other basis.

The sterility noted by Beard in female mice was characterized by a marked decrease in the number of litters produced in comparison with mice maintained on the stock diet. Pregnancy was based upon weight changes in the animal and was not verified by examination of the vaginal smears. Furthermore, the sterile mice were not examined for gross or histological evidence of fetal resorption. At the time these studies were made little was known concerning the nature of this latter process which has since been so thoroughly studied in the rat (Evans and Burr, 1927). Sterility in the males was characterized by infrequency of fertile matings when placed with normal females, and by degenerative changes observed in the germinal epithelium of the testis. It should be stated that one of us (K. E. M.) had the privilege of examining histological preparations of

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the testes from these mice and, at that time, was of the opinion that the injury observed resembled that encountered in the vitamin E deficient rat. However, the evidence was not conclusive on this point and was not supported by attempts to repair the testicular damage which, in the light of later knowledge, would have offered a ready means of determining whether lack of vitamin E was the limiting factor. It was felt that a reinvestigation of reproductive functions in mice would constitute a definite contribution to the limited knowledge concerning vitamin E requirements of different animal species.

Experimental. Mice of the breeding colony were maintained on a diet of commercial dog biscuit known to afford about three times the minimal daily requirement of vitamin E for the rat. At the 10th day of lactation mothers and their progeny were transferred to the vitamin E deficient diet, upon which the young mice were maintained after weaning at the 21st day of age. The mice were housed in metal cages with wood shavings, except for one series of males reared in wire cages with raised screen floors such as employed in our studies with rats. The composition of the deficient diet used, and data concerning the effectiveness of this same dietary procedure in inducing early sterility in male and female rats, are presented in the following report (Mason, 1940). The experimental female mice, mated with normal stock males as soon as sexual maturity was attained, were successfully inseminated at an average age of 70 days. Vaginal smears were made daily prior to mating and at frequent intervals during pregnancy. Testes and epididymides, obtained from experimental males by operation or at autopsy, were routinely fixed and sectioned for histological examination.

Results. Our experiences in producing, and in preventing, sterility in mice under these conditions are summarized in table 1. It will be noted that the females consistently resorbed during their first pregnancy (groups 1 to 3) and that the sterility could be prevented by administration of fresh raw wheat germ, or wheat germ oil, during early stages of the second pregnancy (group 3). First pregnancy resorptions were readily prevented by administering a concentrate from wheat germ oil (group 4) and synthetic alpha tocopherol (group 5) during the first 10 days of pregnancy. Female mice reared on a modification of the E-deficient diet, in which 5 per cent of the lard was replaced by an equivalent amount of hydrogenated cottonseed oil (Crisco), representing one-fourth the concentration of the latter in the diet used by Beard, permitted delivery and lactation of normal litters (group 6). It is of interest that the minimal amount of wheat germ oil concentrate, and of synthetic alpha tocopherol, required to prevent resorption in mice was approximately one half that required by female rats (table 1, footnotes).

When mice of the breeding stock were maintained on diets containing

relatively large amounts of vitamin E, and the litters given access to this diet during lactation, storage of the vitamin in the newly weaned females

TABLE 1

Reproductive behavior of mice of both sexes reared and maintained on a diet deficient in vitamin E

GROUP	NUMBER AND SEX OF MICE	REPRODUCTIVE PERFORMANCE
1	6 females	Resorbed during first and second pregnancies. The second resorption verified by autopsy on the 16th day
2	12 females	Resorbed during first pregnancy. Verified by autopsy on the 16th day
3	14 females	Resorbed during first pregnancy. Fertility restored by feeding wheat germ, or wheat germ oil, during early stages of the second pregnancy
4	14 females	Fed concentrate from wheat germ oil at first pregnancy:* 0 mgm.—3 neg. 15 mgm.—2 neg. and 3 pos. 20 mgm.—3 pos. 25 mgm.—3 pos.
5	20 females	Fed synthetic alpha tocopherol at first pregnancy:† 0 mgm.—5 neg. 0.125 mgm.—2 neg. 0.25 mgm.—1 neg. and 1 pos. 0.35 mgm.—3 pos. 0.50 mgm.—3 pos. 0.70 mgm.—3 pos. 1.0 mgm.—2 pos.
6	7 females	5% of hydrogenated cottonseed oil (Crisco) included in -E diet. 1st pregnancy resulted in delivery of normal litters, successfully suckled
7	40 males	18 on experiment for 100-200 days 10 on experiment for 200-300 days 12 on experiment for 300-400 days } Testis and epididymis histologically normal in all cases

* Fed in a single dose on the 4th day of pregnancy, as an ether solution evaporated on a small amount of dextrin. Thirty milligrams of this concentrate represented the mean fertility dose (i.e., that giving a positive response in 50 per cent of bio-assay tests) for female rats.

† Supplied through the courtesy of Merck and Co., Inc., Rahway, N. J. The tocopherol was administered orally as an olive oil solution, the total dose being distributed over the first 10 days of pregnancy. Bio-assay tests reported elsewhere (Mason, 1940; table 4) indicated that 0.7 mgm. of this tocopherol represented close to the mean fertility dose for female rats. In groups 4 and 5, a positive response implied either the presence of two or more living fetuses at the 16th day of pregnancy or the delivery of litters at full term.

was sufficient to permit one or two pregnancies before the occurrence of a typical resorption gestation. This "first litter fertility" occurs in rats under similar conditions. The histopathological changes observed in the

resorption sites resembled in every way those encountered in the E-deficient rat. The same was true of implantation sites containing dead fetuses, several of which were encountered in mice given critically low doses of vitamin E and autopsied during the last week of pregnancy. This phenomenon of late fetal death, which we have frequently observed in rats used for bio-assay tests and autopsied at the 16th day of pregnancy, will be discussed more fully in a later communication. The neuromuscular disorder so frequently seen during late stages of lactation in the progeny of female rats possessing a critically low storage of vitamin E was not observed in any of our suckling mice. It is possible that the level of vitamin E depletion necessary for the appearance of this phenomenon was not attained in our experiments. On the basis of the evidence presented above, it can be assumed that the female mouse responds qualitatively to vitamin E depletion and to vitamin E therapy in the same manner as does the female rat, and manifests the same histopathological alterations in the fetus and fetal membranes.

On the other hand, our efforts to induce sterility in male mice have met with complete failure. Forty male mice, mostly littermate brothers of the females described above, were prepared and reared in exactly the same manner. After 100 to 400 days of experimental feeding (group 7; table 1) the testes and epididymides from each mouse were examined histologically. In no instance was there any evidence of testicular degeneration. These findings are quite contrary to our experiences with male rats in which the same experimental procedure consistently produces histopathological changes in the seminiferous epithelium after an experimental period of 35 to 50 days (Mason and Bryan, 1940; Mason, 1940).

Discussion. It seems difficult to escape the conclusion that the vitamin E requirements of the male mouse, if the latter can be shown to be dependent upon an extraneous source of vitamin E, must be very much less than those of the female mouse. We were at one time of the opinion that such a sex difference in vitamin E requirements existed in the case of the rat, but have since been able to demonstrate that the minimal daily intake necessary to maintain reproductive functions in the male and female rat is essentially the same and have attributed this apparent sex difference in E-requirements to age differences in the manifestation of urgent needs for the vitamin in the two sexes (Mason, 1940). These qualitative differences are of such a nature that sterility should be more readily produced in males than in females if the presence of traces of vitamin E in the experimental diet were in any way related to the striking differences observed in the response of male and female mice to deprivation of vitamin E. Unfortunately, the experimental data presented in this report offer no adequate explanation for the latter phenomenon.

SUMMARY

1. Female mice reared on a purified vitamin E deficient diet exhibited resorption of the fetuses which, on the basis of gross and histological examination of the placental sites, was indistinguishable from that observed in the vitamin E deficient rat. Sterility was prevented by feeding natural sources of vitamin E or synthetic alpha tocopherol.

2. The same dietary procedure, which also induces sterility in rats of both sexes at the onset of sexual maturity, failed to produce testicular injury in male mice after an experimental feeding period of 400 days.

3. Good reproduction observed in female mice when 5 per cent of the lard in the deficient diet was replaced by an equivalent amount of hydrogenated cottonseed oil, suggests that the previously reported observations concerning sterility in mice due to inadequate vitamin E (Beard, 1926) are subject to some other interpretation.

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MINIMAL REQUIREMENTS OF MALE AND FEMALE RATS FOR VITAMIN E[1]

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It has been suggested that vitamin E may be bipartite in nature, consisting of separate factors responsible for fertility in the two sexes of the rat (Grijns and Dingemanse, 1933; Grijns, 1939). These conclusions were based upon the observation that concentrates obtained from wheat germ oil, separable on the basis of their solubility in alcohol and benzol, differed in their ability to prevent sterility in male and female rats. Grijns (1939) also observed that certain of his experimental diets caused sterility in males but not in females, while other diets induced sterility in females but not in males, which he interpreted as confirming the existence of separate anti-sterility factors for the two sexes. The observation that the presence of 8 per cent butter (Geller and Schuster, 1934), or of 20 per cent tapioca starch (Martino, 1934), in diets otherwise deficient in vitamin E causes sterility in female rats but not in males has also prompted the suggestion referred to above. Various other investigators have noted that certain diets presumably low in vitamin E sometimes prove more effective in causing sterility in one sex than in the other, the preponderance of opinion indicating that the vitamin E requirements of the male rat are considerably greater than those of the female.

Some experimental evidence obtained in this laboratory has been suggestive of a slightly greater requirement for males than for females. Certain diets treated with ferric chloride in ether have produced an irreversible testis injury in male rats indistinguishable from that of E-deficiency but have permitted continued reproduction in littermate sister rats, while other diets treated in a similar manner have induced sterility in both sexes. Furthermore, purified E-deficient diets containing 20 per cent of winter butter have caused early testis injury in males but no sterility in females, although the same proportion of summer butter in the diet has permitted continued fertility in both sexes (Mason and Bryan, unpublished studies). On the other hand, it has been noted that single doses of wheat germ oil

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concentrates administered to newly weaned rats reared on an E-deficient diet may postpone the onset of testis degeneration beyond the 100th day of life and yet prove ineffective in preventing resorptions in females mated at an average age of 66 days (Mason and Bryan, 1940). The latter observations led us to postulate that the vitamin E requirements of male rats must be considerably less than those of females (Mason, 1939). In accord with this assumption was our inability to induce testis injury in male mice under experimental conditions which readily produced first pregnancy resorptions in females (Bryan and Mason, 1940), suggestive of a much greater difference in sex requirements for vitamin E in this species of rodent. However, we have acquired considerable data indicating that the reported observations on rats suggesting the existence of more than one anti-sterility factor, or marked sex differences in requirements for a single factor, may be explained on the basis of differences in the age of male and female rats at which vitamin E, in the form of initial storage or subsequent acquisition of the vitamin, becomes effective in the prevention of sterility. This report presents experimental evidence concerning such qualitative time differences in vitamin E needs, together with data relating to quantitative requirements of the male and female rat for vitamin E.

Experimental. Rats of the breeding colony were reared and maintained on a stock diet of commercial dog biscuit affording approximately three times the minimal daily requirement of vitamin E. During the last week of the 21 day lactation period the mothers and their litters were transferred to the vitamin E deficient diet. The latter, which was made up at least twice weekly, was composed of commercial casein (20 per cent), cornstarch (48 per cent), lard (18 per cent), salts (4 per cent), brewer's yeast (8 per cent) and cod liver oil (2 per cent). The progeny, whose storage of vitamin E was thus restricted to the limited amount available through placental and mammary transfer, were maintained on the E-deficient diet after weaning. It has been shown that these rats, referred to as our "standardized" rats, are uniformly and critically depleted of vitamin E from the beginning of life, testicular degeneration consistently appearing within 35 to 50 days after weaning (average, 40 days) in the males and fetal resorption invariably occurring in females experiencing their first pregnancy after a similar experimental period (Mason and Bryan, 1938, 1940). This early attainment of sexual maturity in female rats, which are mated at body weights of 150 to 160 grams and usually conceive after an average experimental period of 36 days, is attributed to three factors: 1, restriction of litters to 6 rats; 2, the experimental use of only those which exceed 40 grams in body weight at the 21st day of life, and 3, the improved lactation performance of the mother and stimulated growth in the suckling offspring following their transfer to the concentrated diet.

A. *Qualitative differences in sex requirements for vitamin E.* Despite

the occurrence of testicular degeneration in standardized males in close relation to the onset of sexual maturity, and evidence relative to the severity of vitamin E depletion in these animals from the beginning of life, histopathological changes have not been demonstrable in the germinal epithelium prior to puberty. Since earlier studies (Mason, 1926) had shown that a profound and irreversible physiological disturbance of the germinal epithelium in the E-deficient rat precedes by some days the first appearance of histological damage, it seemed of interest to determine whether the sexually immature testis was affected in like manner. One testis was removed by operation from standardized male rats at intervals of 20 to 40 days on the E-deficient diet (group B, table 1). The operated rats were then given an oral dose of 1 gram of wheat germ oil, transferred to a diet of fresh raw wheat germ, and autopsied at the 60th day of experiment. Representative littermates transferred to the stock diet after weaning, but operated upon and autopsied after similar periods, served as controls (group A, table 1). Histological sections from two portions of each testis, and from the head and tail of the epididymis, were studied microscopically. The data obtained demonstrated that ability of the testis to respond to vitamin E therapy was retained by all rats at the 20th day, was lost by about one half at the 25th day, and was absent in all rats at the 30th day. Similar results were obtained 18 months later in a series of experiments in which the preliminary operative procedure was eliminated (group C, table 1).

During the first 30 days after weaning there were no demonstrable differences in either the growth or the histological state of the seminiferous epithelium of testes from control and experimental rats. Testes removed at the 20th day showed an immature epithelium and the ducts of the epididymis were either empty or contained a few sloughed germ cells. By the 25th day mature spermatozoa were appearing in many of the seminiferous tubules but very few had reached the ducts of the caput epididymis. At the 30th day spermatogenic activity in the testis compared favorably with that seen in the mature testis, although most seminiferous tubules had not attained adult dimensions. The ducts of the epididymis were moderately distended with spermatozoa, and scattered germ cells sloughed from the germinal epithelium of the testis were usually encountered in the more distal ducts of the organ.[2] Not until the 35th and 40th days did the E-defi-

[2] Small numbers of incompletely differentiated cells are normally sloughed from the germinal epithelium and appear in the ducts of the epididymis prior to the beginning of spermatogenic activity. Sometimes the first crop of spermatozoa which appear in the epididymal ducts, intermingled with these cells, also appear atypical in structure. Consequently, it is sometimes difficult to discriminate between these latter changes, which are of normal occurrence, and similar changes which immediately precede and accompany the first demonstrable evidence of E-deficiency degeneration.

cient rats begin to show histological evidence of testicular degeneration (in slightly more than half the animals in the two groups) although irreparable damage had been induced in the majority of rats at the 25th day of feeding.

TABLE 1

Showing the stage of experimental feeding at which the testes of standardized male rats lose their ability to respond to vitamin E therapy

Diet after weaning, days	No. of rats	Body wt. in gm., aver.	Testes removed at operation: Wt. in gm., aver.	Testes removed at operation: Per cent normal,* aver.	Testes removed at operation: Histology†	Diet after weaning, days	No. of rats	Body wt. in gm., aver.	Testes removed at autopsy: Wt. in gm., aver.	Testes removed at autopsy: Per cent normal,* aver.	Testes removed at autopsy: Histology†
Group A											
S20	4	116	0.585	80	Normal	S60	4	251	1.46	114	Normal
S25	4	133	0.806	98	Normal	S60	4	237	1.43	120	Normal
S30	6	155	1.063	113	Normal	S60	4	234	1.52	128	Normal
Group B											
−E20	8	114	0.670	92	Normal	−E20+E40	8	244	1.54	127	Normal
−E25	11	148	0.887	101	Normal	−E25+E35	4	253	1.50	124	Normal
							7	251	0.84	68	2 (P-P); 5 (4-5)
−E30	10	188	0.975	113	Normal	−E30+E30	6	247	0.62	51	6 (4-5)
−E35	7	200	1.18	109	4 (N); 3 (N?-1)	−E35+E25	7	251	0.79	66	2 (P-P); 2 (2-4); 3 (4-5)
−E40	8	221	0.97	90	3 (N); 2 (N?-1); 2 (1); 1 (2-3)	−E40+E20	4	233	0.83	71	4 (4-5)
Group C											
						−E20+E40	5	261	3.02 (2)	128	Normal
						−E25+E35	5	257	2.99 (2)	120	Normal
							7	260	2.21 (2)	88	7 (P-P)
						−E30+E30	6	257	1.68 (2)	66	1 (N); 1 (P-P); 4 (4-5)

* Based on the figures of Donaldson (1924) for normal testis weight in relation to body weight.

† The stage, or stages, of degeneration predominating in the testes are indicated in parentheses, and are based upon the arbitrary divisions of the degenerative process described in an earlier report (Mason, 1926). The designation P-P refers to the condition of "partial prevention," characterized by the occurrence of completely degenerated seminiferous tubules intermingled with variable numbers of normal tubules in which the germinal epithelium, having suffered no irreparable injury at the moment vitamin E therapy became effective, was protected against degenerative changes.

These observations indicate that standardized male rats manifest a critical need for vitamin E within 20 to 25 days after weaning (41 to 46 days of age) in order to prevent an irreversible physiological disturbance in the germinal epithelium which precedes, by at least 10 days, the appearance of histopathological alterations. On the other hand, the vitamin E needs of the female rat for reproductive success are of a distinctly different

order. These needs arise only after conception occurs, can be satisfied at any time during the first week of pregnancy, and recur periodically throughout the reproductive period. Under optimum conditions for rapid growth and early sexual maturity, conception in females usually follows by at least two or three weeks the time at which males manifest their critical needs for vitamin E. There is also some evidence (Mason and Bryan, 1940) that the female requires during the first week of pregnancy a relatively greater concentration of vitamin E to prevent fetal resorption than would be required by males at their critical stage in order to postpone the onset of testis degeneration for a period comparable to the life of the developing fetus. It is thus apparent that if rats of both sexes possess the same initial storage of vitamin E at weaning, and are subsequently reared on an E-deficient diet, the males will be able to take better advantage of this storage than the females in whom much of the original supply may be dissipated by general metabolic processes before it is required for reproductive functions. This is well illustrated by observations presented elsewhere (Mason and Bryan, 1940) demonstrating that one can administer to newly weaned standardized rats of both sexes amounts of vitamin E sufficient to postpone the onset of testicular degeneration beyond the 100th day of life and yet fail to prevent resorption in females inseminated at an average age of 66 days.

On the basis of what has just been said it is conceivable that under experimental conditions characterized by an appreciable storage of vitamin E in newly weaned rats, with or without the presence of traces of E in the diet, the males might be able to maintain testis normality up to and beyond a time when sterility was demonstrable in females. On the other hand, under conditions characterized by a negligible storage of E in newly weaned rats subsequently reared on a diet containing traces of the vitamin, the males might not acquire sufficient vitamin by the 40th to 45th day of age to ward off testicular damage although females might eventually store enough to permit the completion of gestation. The latter situation would naturally be accentuated by any dietary increment in vitamin E subsequent to the period at which the needs of the male become critical. Failure to realize these qualitative differences in vitamin E requirements for the rat may explain why investigators have been led to suggest the existence of separate factors, or the need for widely different amounts of the same factor, for the prevention of sterility in the two sexes. In an effort to determine whether male and female rats actually differ quantitatively in their requirements for vitamin E, the following investigations were undertaken.

B. *Quantitative requirements for vitamin E.* Standardized male and female rats were given small daily doses of vitamin E in the form of a concentrate from wheat germ oil and synthetic alpha tocopherol, diluted

with pure olive oil such that each daily dose involved the administration of 0.05 to 0.20 cc. of solution, fed orally by means of small tuberculin syringes. The absence of vitamin E in olive oil had been demonstrated by the sterility consistently observed in standardized rats reared on an E-deficient diet containing 20 per cent of this oil as the fat constituent of the ration. The stock solutions and the small portions removed as needed for feeding were kept at 0°C. Daily dosage was begun on the 10th day after weaning, at which time the rats had an average body weight of 80 grams, and was continued throughout the experimental period except in a few instances as indicated in the summarized data (tables 2 and 3).

Males. Thirty two male rats were given graded doses of the two preparations. In view of extensive data already available in this laboratory concerning the onset of testis injury in the standardized male rats the untreated controls were limited to 6 rats, all of which responded in typical manner. One testis was removed by operation at the time of expected onset of degenerative changes, the other testis being taken at autopsy 5 to 20 days later. A study of stained smears from the testis and from the head and tail of the epididymis, obtained at the time of testis removal, afforded a preliminary analysis of the extent of injury. Representative sections from each testis and epididymis were subsequently examined histologically and the testes classified according to the predominant state or stages of degeneration present (Mason, 1926), the epididymis serving as a useful index of recent events in the seminiferous epithelium. Correlation of these observations with data obtained concerning the time intervals between the different stages of degeneration permitted a relatively accurate estimate of the date of onset of histopathological injury. The results of these studies are summarized in table 2.

Daily doses of 0.0625, 0.125, 0.25, 0.5 and 1 mgm. of the wheat germ oil concentrate effected a significant delay in the onset of testis injury, which became more apparent at increased levels of dosage, but eventually failed to afford protection. Doses of 2 mgm. daily proved adequate throughout the experimental period of 80 days, although cessation of dosage at the 60th day of experiment resulted in the onset of degeneration within two weeks. Daily doses of 4 mgm. afforded adequate protection throughout a 90 day period, even when dosage was discontinued at the 70th day. It appears that a daily intake of 2 mgm. of this concentrate can be regarded as being close to the minimum for maintaining structural integrity of the testis in the standardized male rat.

A daily intake of 0.0375 mgm. of alpha tocopherol effected an average delay of only 11 days in the onset of testis injury, although twice this level of intake (0.070 mgm.) afforded complete protection throughout the experimental period. In the one rat continued to the 90th day, the daily dose was discontinued at the 70th day as a check on the extent of vitamin E

TABLE 2

Showing the effectiveness of a concentrate from wheat germ oil and of alpha tocopherol, administered in daily doses from the 10th day after weaning, upon the onset of testis degeneration in standardized male rats

DAILY DOSE	TOTAL DOSE	TESTIS AND EPIDIDYMIS REMOVED				DAYS ON EXPER. AT ONSET OF TESTIS DEGENERATION (ESTIMATED)	
		At operation		At autopsy			
		Da. on exper.	Stage of degen.	Da. on exper.	Stage of degen.		
Controls							
mgm.	*mgm.*						aver.
		50	4–5	55	5	37	
		40	N	50	N?–1	48	
0	0	40	1–2–3	50	4–5	34	40.5
		40	N	50	1	46	
		40	N–N?	50	3–4	39	
		40	N?–1	50	3–4	38	
E-concentrate*							
0.0625	2.8	50	2–4	55	4–5	42	43.0
0.0625	2.8			55	1–5	44	
0.125	5.6	50	4	55	5	40	45.5
0.125	5.6			55	1–2	51	
0.25	11.2	50	1–2–3	55	2–5	44	
0.25	11.2			55	3–4	47	47.2
0.25	11.2	50	N–N?	55	1–2	50	
0.25	11.2	50	N?–1	55	2–3	48	
0.50	25	60	2–4	70	5	52	
0.50	25	55	1	60	2–3	52	53.5
0.50	25	55	N	60	1	57	
0.50	25	55	N?–1	60	2–3	53	
1.0	60	60	N	70	2–3–4	62	
1.0	50	55	N–N?	60	1–2	55	56.2
1.0	50	55	N–N?	60	1–2–3	54	
1.0	60	60	1–2–3	70	4–5	54	
2.0	100	70	3–4	80	5	62‡	
2.0	100	60	N	70	N?–1	68‡	
2.0	100	60	N	80	1–2–3	74‡	
2.0	120	60	N	80	N		
2.0	140	60	N	80	N		

* A molecular distillate of wheat germ oil kindly supplied by the Research Laboratories of General Mills, Inc., Minneapolis, Minnesota.

† Synthetic alpha tocopherol supplied through the courtesy of Merck and Co., Inc., Rahway, N. J.

‡ Daily dose discontinued after the 60th day of experiment.

** Daily dose discontinued after the 70th day of experiment.

TABLE 2—*Concluded*

DAILY DOSE	TOTAL DOSE	TESTIS AND EPIDIDYMIS REMOVED: At operation: Da. on exper.	TESTIS AND EPIDIDYMIS REMOVED: At operation: Stage of degen.	TESTIS AND EPIDIDYMIS REMOVED: At autopsy: Da. on exper.	TESTIS AND EPIDIDYMIS REMOVED: At autopsy: Stage of degen.	DAYS ON EXPER. AT ONSET OF TESTIS DEGENERATION (ESTIMATED)
			E-concentrate*—*Concluded*			
mgm.	*mgm.*					*aver.*
4.0	280	70	N	80	N	
4.0	200	70	N	90	N	
4.0	200	70	N	90	N	**
			Alpha tocopherol†			
0.0375	1.7	50	N	55	N–N?	54
0.0375	1.7	50	N?	55	1	51 } 51.5
0.0375	1.7	50	N	55	N?–1	53
0.0375	1.7	50	N?–1	55	2–3	48
0.075	4.5	60	N	70	N	
0.075	4.5	60	N	70	N	
0.075	5.25	60	N	80	N	
0.075	6.0	70	N	90	N	**

storage at that period. It would seem that the minimal daily intake of alpha tocopherol for standardized males lies somewhere between these two levels of dosage.

Females. Forty female rats, similarly fed, were mated with normal males when they had reached body weights of 150 grams or better, and their reproductive activity followed by daily vaginal smears. Conception occurred after an average experimental period of 45 days despite the delay caused by 9 instances of pseudopregnancy. The daily dose of vitamin E was discontinued after the 10th day of pregnancy in all rats. In most instances laparotomy was performed on the 16th day of pregnancy to record the distribution of living fetuses, dead fetuses and resorption sites for subsequent comparison with results obtained at term. At the time of expected delivery the rats were placed upon a coarse wire screen to permit the young to fall out of reach of the mother after delivery. The data obtained are summarized in table 3.

The concentrate from wheat germ oil consistently gave negative responses at a daily intake of 1 mgm. or less, and positive responses at an intake of 4 mgm. or more. A daily intake of 2 mgm. enabled 7 out of 10 rats to show a positive response. The occurrence of dead fetuses in four of these rats also demonstrates the critical character of this level of intake. It is to be noted that the responses elicited by this concentrate, and by the alpha tocopherol, were not always proportional to the total doses administered. This is apparently due to biological variations in animal response

which become particularly evident at critical levels of dosage. Especially significant is the observation that a daily intake of 2 mgm. of the concentrate proved to be close to the minimum for preventing sterility in both

TABLE 3

Reproductive performance of standardized vitamin E deficient rats given small daily doses of 1, a concentrate of wheat germ oil, and 2, synthetic alpha tocopherol, fed orally from the 10th day after weaning to the 10th day of their first gestation period

DAILY DOSE	TOTAL DOSE	UTERUS AT 16TH DAY L. D. R.	DAILY DOSE	TOTAL DOSE	UTERUS AT 16TH DAY L. D. R.
		E-concentrate			
mgm.	*mgm.*		*mgm.*	*mgm.*	
0.25	11	0 -0- 9	2.0	72	7 -0- 1 (7)*
0.25	14	0 -0-10	2.0	72	8 -0- 0 (7)
0.25	12	0 -0- 8	2.0	74	7 -2- 0 (5)
0.25	12	0 -0-10	2.0	90	2 -3- 3 (1)
			2.0	82	3 -2- 4 (3)
0.50	23	0 -0-11	2.0	94	6 -0- 2†
0.50	25	0 -0- 7	2.0	90	2 -1- 7†
0.50	22	0 -0-10			
0.50	22	0 -0- 7	4.0	104	6 -0- 3 (4)
			4.0	104	6 -0- 1 (6)
1.0	47	0 -0-11	4.0	144	9 -0- 2†
1.0	49	0 -0- 9	4.0	220	7 -0- 1 (7)
1.0	47	0 -0- 9	4.0	224	8 -0- 5†
1.0	43	0 -0- 9			
			8.0	296	—— (8)
2.0	96	0 -0-11	8.0	424	—— (8)
2.0	98	0 -0-11	8.0	440	—— (8)
2.0	108	0 -0-10			
		Alpha tocopherol			
0.0375	1.38	0 -0-10	0.075	2.92	7 -0- 3 (7)
0.0375	1.87	0 -0-10	0.075	3.00	8 -0- 1 (8)
0.0375	2.32	0 -0- 6	0.075	3.00	8 -0- 1 (7)
0.0375	1.95	4 -1- 4 (2)	0.075	3.45	10 -0- 1 (10)
0.0375	2.14	8 -1- 2 (9)	0.075	5.32	8 -0- 2 (8)

* Figures in parentheses indicate the number of offspring delivered at full term.
† Autopsied on 16th day.

sexes. Concurrent bio-assay tests (table 4) indicated that the "mean fertility dose"[3] of this concentrate was slightly in excess of 30 mgm., probably

[3] The term "mean fertility dose," first suggested by Bacharach (1938), denotes the dose capable of giving a positive response in 50 per cent of bio-assay tests It must be kept in mind that the response given at critical levels of dosage may be appreciably modified by differences in the period over which the test dose is administered, as shown by the data in table 4. In this laboratory, administration of the test dose as five equal doses on the 4th to 8th days of pregnancy has proved to be a very satisfactory procedure for general use. The advantages of the 16 day autopsy procedure for evaluating responses will be discussed in a later report.

about 34 mgm., which would represent between fifteen and twenty times the minimal daily dose. It is of interest that this latter dose (2 mgm.) is essentially that calculated by Bacharach (1938) who states "If M be the mean fertility dose found for a substance administered over 10 days to virgin animals about 100 days old, presumably a daily dose of 6M/100 would be quite adequate if given daily from birth, or even from weaning."

The doses of alpha tocopherol selected for daily feeding represented 1/10th and 1/20th of 0.7 mgm. which, according to the recent observations of Bacharach (1939) and the bio-assay data presented in table 4,

TABLE 4

Results of bio-assay tests on the concentrate from wheat germ oil, and the synthetic alpha tocopherol, used in the studies presented in tables 2 and 3

The presence of two or more living fetuses at autopsy on the 16th day of the first pregnancy was taken as a criterion of positive response.

CONCENTRATE FROM WHEAT GERM OIL				ALPHA TOCOPHEROL	
Fed as 5 equal doses on the 4th to 8th days of pregnancy			Fed as 5 equal doses on the 4th day of pregnancy	Fed as 10 equal doses on the 1st to 10th days of pregnancy	Fed as 10 equal doses on the 4th day of pregnancy
Total dose, 20 mgm.	Total dose, 30 mgm.	Total dose, 50 mgm.	Total dose, 50 mgm.	Total dose, 0.7 mgm.	Total dose, 0.7 mgm.
L. D. R.	L. D. R.	L. D. R.	L. D. R.	L. D. R.	L. D. R.
0 -0- 6	0 -0- 5	9 -0- 1	9 -0- 1	10 -0- 0	2 -2- 8
0 -2- 4	0 -1- 0	11 -0- 0	10 -0- 0	8 -0- 1	1 -1- 9
0 -0-10	8 -0- 1	11 -0- 0	12 -0- 0	8 -1- 0	0 -0-11
0 -0- 3	0 -2- 6	8 -0- 1	1 -1-11	7 -0- 0	0 -0-10
0 -0- 8	1 -1- 5	9 -0- 0	3 -0- 6	7 -0- 1	0 -5- 5
0 -0- 8	0 -1- 7	8 -0- 0	6 -0- 3	4 -2- 4	2 -4- 5
0 -0- 7	0 -2- 8	7 -0- 2	5 -0- 3	3 -0- 2	9 -1- 1
3 -4- 2	4 -0- 5	7 -0- 0	3 -1- 4	1 -0- 9	4 -0- 7
0 -0-10	8 -2- 0	7 -0- 0	10 -0- 0		
0 -0-12	6 -2- 2	6 -0- 4	8 -0- 1		
		10 -0- 0			
10%+	40%+	100%+	90%+	87.5%+	50%+

approximated the mean fertility dose. The results obtained compared favorably with those in the studies with the wheat germ oil concentrate. A daily intake of 0.0375 mgm. sufficed to prevent resorption in 2 of the 5 female rats tested (table 3). The occurrence of dead fetuses in both rats indicates the critical character of this level of dosage which, as will be recalled, did not afford sufficient protection for males. On the other hand, twice this daily intake (0.070 mgm.) proved more than adequate for completion of gestation in females and for maintaining an intact seminiferous epithelium in males. It can be assumed that the minimal protective dose lies between these two levels of intake. Although the data pre-

sented are too limited to permit a conclusive statement concerning the alpha tocopherol requirements of male and female rats, it is apparent that the needs of the two sexes for the synthetic vitamin are very similar. Furthermore, the ratio between the minimal daily dose and the mean fertility dose corresponds closely to that observed in the experiments with wheat germ oil concentrate.

Discussion. Throughout these studies no allowance has been made for possible variations in relationship between the age or the body weight of rats and their vitamin E requirements. The results of bio-assay studies carried out in this laboratory indicate that the reproductive needs of the female are not significantly influenced by differences in body weight. Since the males had attained body weights of 350 to 400 grams by the end of the 90 day experimental period, it does not seem likely that their needs for vitamin E would have increased appreciably had they been continued on experiment for a longer period. It is possible, however, that the lesser effectiveness of the lower level of tocopherol dosage (0.0375 mgm.) in males than in females may be attributed to the relatively larger size of the former sex. Calculated on the basis of the mean body weight of both sexes during the period over which the doses were administered, the males and females fed the fully protective dose of 0.070 mgm. received, respectively, 0.33 and 0.48 mgm. of tocopherol daily, per kilogram of body weight. This represents one-third to one-half the amount of alpha tocopherol, in terms of milligrams per kilogram of body weight, which Mackenzie and McCollum (1940) have estimated to be necessary for protecting rabbits against muscular dystrophy.

Since such very different structures (developing fetus and seminiferous epithelium) are affected by deprivation of vitamin E in the two sexes, it is indeed remarkable that the daily requirements of males and females for the two preparations used have proved so similar. Although we have no information on the tocopherol content of the wheat germ oil concentrate used in these studies, it is generally accepted that wheat germ oils produced in this country contain several times as much alpha tocopherol as beta tocopherol, and probably contain no other biologically active tocopherol. Consequently, the studies presented in this report have been concerned primarily with the minimal requirements of the rat for alpha tocopherol, in its natural and synthetic forms. It is hoped that similar studies may eventually be extended to an analysis of the requirements of male and female rats for the beta and gamma forms of tocopherol, and for chemically related compounds possessing biological activity characteristic of vitamin E.

Our observations confirm those of Evans, Emerson and Emerson (1939) concerning the ability of synthetic alpha tocopherol to function as vitamin E in the male as well as in the pregnant female. On the assumption that the male rat requires considerably more vitamin E than the female.

these investigators fed daily doses of 80 mgm. of wheat germ oil to five male rats and 0.75 mgm. of alpha tocopherol to six other males, otherwise deprived of vitamin E, from the 21st to the 277th day of life. On the basis of their data and our experimental findings, their rats received approximately three and fifteen times the minimal requirement of wheat germ oil and tocopherol, respectively. Although the testes were normal in both groups, the rats fed wheat germ oil showed defective sex interest. This is in keeping with the lower intake of E in this group and suggests that the vitamin requirements for functional normality of the male rat may be somewhat greater than for maintenance of a normal seminiferous epithelium. Unfortunately, no mating tests were carried out on the males of our series. Changes in the interstitial tissue of the testis and in the epithelial lining of the accessory sex glands, which Evans et al. (1939) describe as being particularly marked in their tocopherol fed rats, were not observed in our animals. It seems unlikely that the shorter period of experimental feeding in our studies can account for these differences in observation. It is also of interest that Koneff (1939), reporting on the cytological characteristics of the anterior pituitary glands of the rats used in Evans' study, states that "in the majority of the respects studied there was an improvement toward normality in the following order: E-low → alpha tocopherol → wheat germ oil → natural food." These findings are nót in accord with the levels of vitamin E intake in the different groups. Further investigations are necessary before it can be stated with certainty that synthetic alpha tocopherol can completely replace vitamin E from natural sources. The studies reported here indicate that the synthetic and natural forms of tocopherol are about equally effective in permitting normal reproduction in the female rat and in maintaining structural integrity of the reproductive system in the male.

SUMMARY AND CONCLUSIONS

1. Male rats deprived of vitamin E from the beginning of life require a definite source of the vitamin prior to the 40th to 50th days of age in order to prevent irreversible physiological disturbances in the germinal epithelium which antedate, by 10 days or more, the onset of histopathological changes; whereas the reproductive needs of the female rat for vitamin E arise subsequent to conception and implantation, which may occur at any stage of the reproductive period.

2. It is emphasized that these qualitative differences in sex requirements for vitamin E must be taken into consideration in the evaluation of experimental data interpreted as demonstrating the existence of separate anti-sterility factors, or the need of different amounts of a single factor, for the prevention of sterility in male and female rats reared on diets deficient in vitamin E.

3. The daily administration of 2 mgm. of a concentrate from wheat

germ oil fed orally from the 10th day after weaning to rats depleted of vitamin E from early life proved equally effective in preventing sterility in male and female rats, but daily doses of 1 mgm. or less were inadequate.

4. Similar administration of 0.0375 mgm. of synthetic alpha tocopherol caused a slight delay in the onset of testicular degeneration in males and permitted 2 out of 5 females to complete gestation; while twice this dose proved more than adequate for the prevention of sterility in both sexes. The minimal protective dose must lie between these two levels of intake.

5. It is concluded that the minimal daily requirements of vitamin E (as wheat germ oil concentrate or as synthetic alpha tocopherol) for preventing sterility in E-deficient rats are essentially the same for both sexes and, on the basis of bio-assay tests, represent between one-fifteenth and one-twentieth the mean fertility dose for adult female rats.

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ON THE VAGO-INSULIN AND SYMPATHETICO-ADRENAL SYSTEM AND THEIR MUTUAL RELATIONSHIP UNDER CONDITIONS OF CENTRAL EXCITATION INDUCED BY ANOXIA AND CONVULSANT DRUGS[1]

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McQuarrie, Ziegler, Wangensteen and Dennis (1939) showed recently that whereas anoxia produces hyperglycemia in normal dogs it causes a fall in blood sugar in adrenalectomized animals. In order to explain this interesting phenomenon we have repeated their experiments in rabbits and rats and analysed them further by studying the rôle of the splanchnics and of the vagi. The experiments seem to indicate that under the influence of anoxia both parasympathetic and sympathetic centers are excited. Evidence is presented to show that the secretion of insulin may be stimulated by the action of oxygen lack on autonomic centers.

It seemed to us of interest to supplement these investigations by studying under similar conditions the action of a drug which is known to produce marked sympathetic effects by central stimulation of autonomic centers. Metrazol was chosen because it increases the excitability of sympathetic hypothalamic centers as measured by the height of contraction of the nictitating membrane, thus acting similarly to anoxia (Carlson, Darrow and Gellhorn, 1940). Moreover, Gellhorn and Darrow (1939) showed that intravenous injection of metrazol in convulsive and subconvulsive doses may lead to prolonged discharges from the nictitating membrane (n.m.). The analysis of the pupillary changes showed a marked dilatation due to inhibition of the parasympathetic tone and excitation of the sympathetic; but a temporary phase of pupillary constriction could also be observed in the sympathectomized eye. Metrazol like anoxia causes a sympathetico-adrenal discharge resulting in hyperglycemia. This rise in blood sugar is prevented by splanchnicotomy (Bömer, 1930).

The experiments reported in this paper show that the similarity of

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the action of anoxia and metrazol goes still farther since both factors elicit insulin secretion via the vagi.

METHODS. Anoxia was induced by the inhalation of 7 per cent oxygen for two hours in rats and for three hours in rabbits. No narcosis was employed. Concerning the technic, compare Gellhorn and Packer (1940). The rabbits were first subjected to a typical anoxia experiment. Then the adrenal were denervated bilaterally and the anoxia experiment was repeated after an interval varying from 7 days to 4 weeks.

Forty-nine experiments were performed on rats. These were divided into three groups. The first group comprised the controls which inhaled 7 per cent oxygen for two hours, the second group of experiments was performed on adrenalectomized rats which had been operated on three to seven days earlier and the third group consisted of rats in which not only the adrenals had been removed but which had also been vagotomized below the diaphragm. The blood sugar was determined prior to the experiment and after a period of two hours of anoxia.

A similar group of experiments was performed on rats in which instead of anoxia the effect of 55 mgm. metrazol per kilogram injected subcutaneously was studied. The blood sugar was determined in intervals of 15 and 30 minutes. All animals were starved for 18 hours. The blood sugar was determined by means of the Somogyi modification of the Shaffer-Hartman method. The adrenalectomized animals were allowed to drink a 2 per cent solution of NaCl.

RESULTS. It has been found in numerous experiments that the inhalation of 7 per cent oxygen causes the blood sugar to rise regularly in unanesthetized rabbits (Gellhorn and Packer, 1939). If, however, the adrenal glands were denervated prior to the experiment anoxia no longer caused a rise in blood sugar, but produced uniformly a mild hypoglycemia (fig. 1). Since in these rabbits the secretion of cortin is not interfered with it is obvious that the alteration in response of the blood sugar to anoxia is not due to a deficiency in the cortical adrenal hormone.

After it had been established that the response of the blood sugar to anoxia in animals with denervated adrenals is the same as that found by McQuarrie and collaborators in adrenalectomized dogs, further experiments were conducted on the effect of anoxia on the blood sugar of normal and adrenalectomized rats and of animals in which in addition to the adrenalectomy the vagi had been sectioned intra-abdominally. The results are illustrated in table 1. They show clearly that normal rats respond to anoxia with the usual increase in blood sugar whereas adrenalectomized rats react with a fall in blood sugar, thus confirming the results of McQuarrie in adrenalectomized dogs. The third series of experiments in which in addition to the elimination of the adrenals the vagi had been divided intra-abdominally is particularly interesting.

It was thought that the fundamental difference in the reaction of normal and adrenalectomized rats is due to the fact that in the latter the sympathetico-adrenal system can no longer be stimulated by oxygen lack but this factor may still influence the parasympathetic centers and thereby elicit the secretion of insulin via the vagi. If this hypothesis is correct then it must be assumed that the elimination of the vagi in the adrenalectomized rats would abolish the hypoglycemic effects of anoxia. Table 1 shows indeed that in no instance did a hypoglycemic effect occur in these animals after anoxia. Instead, a slight and statistically insignificant increase of blood sugar is found. The experiments seem to indicate that anoxia stimulates parasympathetic and sympathetic centers at the same time. In the normal animals the stimulation of the sympathetico-adrenal system greatly overbalances the effects

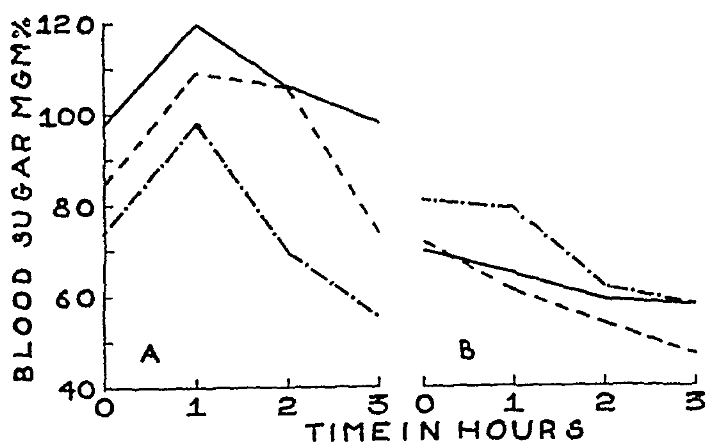

Fig. 1. Effect of inhalation of 7 per cent oxygen on the blood sugar of three rabbits before and after denervation of the adrenals.

on the parasympathetic-insulin system. If, however, the former is removed, the effects on the latter become apparent.

It was of interest to investigate the effect of metrazol on the blood sugar under similar conditions. If metrazol acts on both parasympathetic and sympathetic centers, a reaction similar to that produced by anoxia would call forth a secretion of insulin via the vagi which is normally masked by the more powerful excitation of the sympathetico-adrenal system. Therefore it was to be expected that, whereas metrazol causes a hyperglycemia in normal animals, it would call forth a lowering of the blood sugar in adrenalectomized animals.

Table 2 shows that this is in fact the case. The results are therefore quite comparable to the experiments on anoxia and this holds likewise for the third series of experiments in which the influence of metrazol on the blood sugar was determined in adrenalectomized and vagotomized rats. No statistically significant changes in blood sugar are observed.

The experiments described in this paper show clearly that anoxia and metrazol stimulate the sympathetico-adrenal system to such an

TABLE 1

Effect of 2 hours of anoxia (7% O_2) on normal, adrenalectomized, and adrenalectomized-vagotomized rats

NUMBER	FASTING BLOOD SUGAR	BLOOD SUGAR AFTER 2 HOURS	PER CENT CHANGE
A. Effect on normal rats			
	mgm. per cent	*mgm. per cent*	
1	70.9	78.5	10.7
2	72.0	83.8	16.4
3	75.2	92.5	23.0
4	73.1	75.2	2.9
5	70.9	86.0	21.2
6	72.0	102.1	41.8
7	68.8	88.2	28.2
8	70.9	86.0	21.2
9	69.8	66.6	−4.6
10	69.8	83.8	20.1
Av.	71.3	84.3	18.1
Stand. Dev.	1.75	9.15 $P < 0.01$	
B. Effect on adrenalectomized rats			
1	63.4	41.9	−33.9
2	65.5	43.0	−34.4
3	64.5	40.8	−36.7
4	63.4	46.2	−27.1
5	62.3	40.8	−34.5
6	58.0	41.0	−29.3
Av.	62.8	42.3	−32.6
Stand. Dev.	2.39	1.92 $P < 0.01$	
C. Effect on adrenalectomized-vagotomized rats			
1	60.2	65.6	9.0
2	58.0	60.2	4.0
3	62.3	70.9	13.8
4	61.2	62.3	1.8
5	58.0	55.9	−3.6
6	60.2	60.2	0.0
7	61.2	64.5	5.4
8	59.1	68.8	16.4
Av.	60.0	63.6	5.9
Stand. Dev.	1.49	4.60 $P = 0.04$	

extent in the normal animal that they completely obscure the simultaneous excitation of the vagus which leads to hypoglycemia by means of increased insulin secretion. If this interpretation is correct it should

be possible to show that adrenalectomized animals have more insulin circulating in the blood at the end of the anoxia period than they had

TABLE 2

Effect of metrazol (55 mgm./kgm.) subcutaneously on the blood sugar of normal, adrenalectomized, and adrenalectomized-vagotomized rats

NUMBER	BEFORE METRAZOL	AFTER MERTAZOL		
		15 minutes	30 minutes	60 minutes
		A. Effect on normal rats		
1	70.9	93.5	96.8	99.9
2	70.9	86.0	72.5	70.9
3	72.0	107.5		
4	70.9	88.2	86.0	79.5
5	73.1	107.5	116.1	156.9
6	69.8	78.5	107.5	86.0
7	70.9	101.0	90.3	82.7
8	72.0	96.8	111.8	107.5
9	70.9	104.2	106.4	96.8
10	69.8	110.0	101.0	82.7
Av.	71.12	97.32	99.01	96.0
Stand. Dev.	1.05	3.18 $P < 0.01$	12.03 $P < 0.01$	23.91 $P < 0.01$
		B. Effect on adrenalectomized rats		
1	68.8	43.0	45.1	
2	59.1	51.6	44.0	53.7
3	61.2	50.5	51.6	56.9
4	64.5	50.5	58.0	59.1
5	60.2	52.6		
6	62.3	50.5	51.6	59.1
7	59.1	48.3	46.2	54.8
8	59.1	58.0	49.4	52.6
Av.	61.8	50.62	49.4	56.03
Stand. Dev.	3.19	3.92 $P < 0.01$	4.50 $P < 0.01$	2.53 $P < 0.01$
		C. Effect on adrenalectomized-vagotomized rats		
1	58.0	58.0	72.0	64.5
2	59.1	61.2	61.2	60.2
3	58.0	56.9	61.2	59.1
4	63.4	64.5	63.4	
5	58.0	59.1	73.1	56.9
6	56.9	86.0	72.0	58.0
7	61.2	77.4	83.8	70.9
8	58.0	63.4	62.3	59.1
Av.	59.07	65.81	68.62	61.24
Stand. Dev.	2.02	9.77 $P = 0.09$	7.51 $P < 0.01$	4.53 $P = 0.23$

prior to this experiment. The assay of insulin in very small amounts in the blood is of course a very difficult matter. As a method of assay

the effect of the intraperitoneal injection of blood in adrenalectomized mice was used. Brugsch (1930) has shown that the mouse may be used for the assay of small amounts of insulin. We tried to improve the sensitivity of his method by employing adrenalectomized mice instead of normal mice (cf. Hemmingsen and Nielsen, 1938). As table 3 shows it was found that two hours after adrenalectomized mice had been injected with 0.75 cc. of the blood of adrenalectomized rats the blood

TABLE 3

Assay of blood for insulin using the adrenalectomized mouse as a test animal

The values of blood sugar of adrenalectomized mice 2 hours after the intraperitoneal injection of the blood to be tested

Mice injected with blood from normal, adrenalectomized, anoxic and adrenalectomized, anoxic and adrenalectomized-vagotomized, metrazol treated adrenalectomized, and metrazol treated adrenalectomized-vagotomized rats

1 NORMAL RAT	2 ADRENALECTOMIZED RAT	3 ANOXIC AND ADRENALECTOMIZED RAT	4 ANOXIC AND ADRENALECTOMIZED-VAGOTOMIZED RAT	5 METRAZOL* TREATED ADRENALECTOMIZED RAT	6 METRAZOL* TREATED ADRENALECTOMIZED-VAGOTOMIZED RAT
59.1	62.3	49.4	66.6	48.3	65.5
56.9	62.3	53.7	63.4	44.0	60.2
60.2	60.2	52.6	64.5	55.9	59.1
	61.2	53.7	64.5	46.2	64.5
	59.1	52.6	64.5	43.0	68.8
	62.3	54.8	60.2	47.3	64.5
	60.2	55.9			
	58.0	51.6			
	59.1	52.6			
		51.8			
		49.4			
Av. 58.7	60.5	52.6	64.0	47.5	63.8
Stand. Dev.	1.51	1.91	1.93	4.19	3.26
		$P < 0.01$	$P < 0.01$	$P < 0.01$	$P = 0.02$

* Injected with 55 mgm. metrazol/kgm. subcutaneously.

sugar of the mice averaged 60.3 mgm. per cent. If, however, blood was withdrawn from the adrenalectomized rats at the end of an anoxia period the mice tested showed blood sugars averaging 52.6 mgm. per cent. The difference between the two blood sugars corresponds to that produced by about 0.0015 unit of insulin per cubic centimeter. Although this amount is very small, the consistency of the results seems conclusive proof that during anoxia insulin is being secreted by impulses reaching the pancreas via the vagi.

This conclusion is supported by further experiments in which the

blood of adrenalectomized and vagotomized rats was tested for insulin at the end of the anoxia period. As table 3 shows, there was no indication of an increased insulin concentration in the blood but a slight hyperglycemic effect of doubtful physiological significance occurred.

Similar experiments using metrazol were performed on adrenalectomized rats with and without subdiaphragmatic vagotomy. It was found in agreement with the results on anoxia that the insulin content of the blood of adrenalectomized rats was increased after metrazol but such increase was not found after the adrenalectomized animals had been subjected to sectioning of the vagi.

Discussion. The stimulation of autonomic centers by anoxia is probably exclusively due to the action of oxygen lack on chemoreceptors in the sino-aortic area (cf. Gellhorn and Lambert, 1939) whereas metrazol seems to act directly on the autonomic centers since its action is not interfered with by the removal of the buffer nerves (Gellhorn and Darrow, 1939; Carlson, Darrow and Gellhorn, 1940). It seems therefore not improbable that other drugs and physiological factors which are known to stimulate the sympathetico-adrenal system may at the same time excite the vago-insulin system. This seems in fact to be the case. It is well known that insulin hypoglycemia leads to a stimulation of the sympathetico-adrenal system (Cannon, McIver and Bliss, 1924; Schlossberg, Sawyer and Bixby, 1933; Ernstene, Riseman, Stern and Alexander, 1935; Domm and Gellhorn, 1940; Tietz, Dornheggen and Goldman, 1940). Only in conditions of very severe hypoglycemia, signs of vagal excitation become manifest (Himwich and collaborators, 1939). If, however, indicators are chosen which allow one to study increased parasympathetic activity without the interference of increased sympathetico-adrenal excitation results are obtained which seem to permit of a similar interpretation as was given for the experiments on anoxia and metrazol.

Recent observations of Bender and Siegel on the effect of hypoglycemia on the completely denervated iris indicate the liberation of adrenalin and in some cases of acetylcholine in this condition. With the adrenals intact pupillary dilatation (adrenalin) prevails, but after their removal miosis (acetylcholine) may occur. The authors fail to give an adequate explanation of this interesting phenomenon. In the light of the experiments reported in this paper it seems likely that hypoglycemia, similarly to anoxia, leads to the excitation of sympathetic and parasympathetic centers and that the elimination of the more powerful effects of sympathetic stimulation by removal of the adrenals allows one to recognize the simultaneous effects of parasympathetic excitation which lead to the liberation of acetylcholine.

Some observations of Bodo, Cotui and Benaglia which these authors

were unable to interpret may be adequately understood on the same basis. They found that morphine causes a hyperglycemia in normal cats, a slight increase in blood sugar after denervation of the adrenals but a fall in blood sugar in completely sympathectomized cats. If morphine, like metrazol, excites parasympathetic and sympathetic centers the elimination of the sympathetic system must cause hypoglycemia via the vagi.

On the basis of this discussion it is assumed that not only anoxia and metrazol but also hypoglycemia and morphine produce an excitation of both sympathetic and parasympathetic centers. As far as the balance between the two divisions of the autonomic system is concerned it is definitely shifted toward the sympathetic side in the normal animal. If, however, the sympathetico-adrenal system is eliminated, the alteration in the blood sugar level and its dependence on the integrity of the vagi is proof that anoxia and metrazol (and probably hypoglycemia, morphine and other drugs known to stimulate sympathetic centers) act on sympathetic and parasympathetic centers at the same time. The physiological predominance of the sympathetic over the parasympathetic becomes even more marked when the autonomic centers are stimulated. It seems of considerable interest to point out that central autonomic excitation by anoxia, metrazol (and probably morphine and hypoglycemia) involves both divisions of the autonomic nervous system. Obviously the law of reciprocal innervation does not hold for conditions in which autonomic centers are excited under physiological conditions or by means of drugs.

SUMMARY

1. Whereas inhalation of 7 per cent oxygen causes hyperglycemia in normal rabbits it lowers the blood sugar in rabbits with denervated adrenals.

2. Inhalation of 7 per cent oxygen raises the blood sugar in normal rats and causes hypoglycemia in adrenalectomized rats (confirming the work of McQuarrie and collaborators on dogs). If the vagi are divided intra-abdominally in adrenalectomized rats these animals show either no change in blood sugar or a slight hyperglycemia in response to anoxia.

3. Similar experiments were conducted on the effects of metrazol on the blood sugar in rats. Metrazol causes hyperglycemia in normal and hypoglycemia in adrenalectomized rats. After subdiaphragmatic vagotomy adrenalectomized rats respond to metrazol with no change or a slight rise in blood sugar.

4. The results reported under 2 and 3 are interpreted to mean that anoxia and metrazol cause a stimulation of both the sympathetico-adrenal and the vago-insulin system, the effect on the latter being

masked by the more powerful stimulation of the former. This conclusion is substantiated by the assay of blood of adrenalectomized rats for insulin by using the adrenalectomized mouse as a test animal. It was found that blood obtained from adrenalectomized rats at the end of the anoxia period or after injection of metrazol produced a fall in blood sugar in the test animal. Such a hypoglycemic effect was regularly absent when adrenalectomized and vagotomized animals were subjected to anoxia and metrazol respectively and the blood was assayed for insulin.

5. Since, under conditions of stimulation of autonomic centers with metrazol or (reflexly) by anoxia, parasympathetic and sympathetic centers are activated at the same time it does not seem justified to apply the concept of reciprocal innervation to central autonomic processes.

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THE ADRENAL LIPIDS IN PREGNANT RABBITS

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The adrenal glands are known to change size rapidly under a wide variety of conditions (1) and consequently provide an interesting organ for the comparison of chemical composition and physiological activity. It has long been known that the adrenal gland contains a relatively large amount of lipid and that the lipid fractions are present in unusual proportions. It resembles blood serum rather than other tissues in its lipid proportions, especially in its cholesterol-ester content. For this reason, theories have been proposed that the adrenals actually exert some control over the serum-lipid level; particularly, that the adrenals synthesize or store cholesterol for the maintenance of the blood-serum-cholesterol level. Although it is possible that the adrenal glands play a part in the regulation of the blood-serum level via their hormones, it is highly improbable that they could synthesize or store sufficient lipid to influence appreciably the serum-lipid level.

Except for histological studies, which are difficult to interpret, the relation of adrenal hypertrophy or atrophy to the lipid composition has not been extensively studied. In the adrenal hypertrophy, produced by insulin-hypoglycemic treatments, it was found that phospholipid, neutral fat and total lipid increased in absolute amounts while cholesterol esters and free cholesterol were unchanged (2). When calculated as a percentage of the moist weight, the phospholipid, free cholesterol and total lipid content remained constant while the ester cholesterol decreased and the neutral fat increased in the hypertrophying organ. Cholesterol feeding (3) caused hypertrophy of the adrenals and increase in the phospholipid, free and ester cholesterol, neutral fat and total lipid. Blumenfeld (4) observed that the absolute amount of total lipid in the adrenal glands of spayed rats was decreased in proportion to the degree of atrophy. In the

adrenal hypertrophy of pregnancy and parturition, Anderson and Sperry (5) observed that both the relative and absolute amounts of cholesterol esters, but not free cholesterol, were decreased as compared with those of adrenals in various phases of the oestrus cycle. In addition, the cholesterol esters were decreased in the adrenals of spayed rats. They concluded that cholesterol esters in the adrenal glands are not related to physiological states since spayed animals have decreased and pregnant and parturient rats have increased metabolic processes.

In the present study, we have examined the weights and lipid composition of the adrenals in control, pregnant and pituitary-treated rabbits.

Methods. The methods for the analyses of the adrenal lipids were given in a previous publication (2). The data on the male control rabbits were also given (2). Young female rabbits (3–6 months) were used for the pregnancy and pituitary-injected series. The pituitary preparation was an acetone-insoluble, water-soluble fraction of horse pituitary the activity of which was determined by the production of ovulation. Protocols are given in another publication by Graubard and Pincus (6). The data herein reported were studied for their statistical validity by use of Fisher's "t" test.

$$t = \frac{\bar{X}_1 - \bar{X}_2}{\sqrt{\frac{N_1 + N_2}{N_1 N_2 (N_1 + N_2 - 2)}\left[Sx_1^2 - \frac{(Sx_1)^2}{N_1} + Sx_2^2 - \frac{(Sx_2)^2}{N_2}\right]}}$$

The probabilities attaching to the t's were considered significant when $p < 0.05$.

Results and discussion. The data obtained are presented in tables 1 and 2. It is apparent that the pituitary preparation had no effect on the weight and lipid composition of the adrenals. These results are in agreement with those of Anderson and Sperry (5) that cholesterol and its esters do not vary significantly during various phases of the oestrus cycle.

During the first 6 days of gestation, in which there was no significant change in the weight of the adrenals, the lipid composition changed relatively little; only cholesterol esters and neutral fat were appreciably different. Although the percentage of cholesterol esters was decreased, the absolute amounts were not changed; consequently, the variation in the percentage of cholesterol esters must be attributed to the variations in adrenal weight. A real increase in both relative and absolute amounts of neutral fat occurred.

In the latter part of gestation there was marked increase in adrenal weights and proportionate changes in lipid composition. In the hypertrophied organs there was a significant decrease in phospholipid and cholesterol esters, no change in free cholesterol, and an increase in neutral fat and total lipid. However, the absolute amounts of the various lipid

fractions (calculated as milligrams per kilogram body weight) have increased in the pregnant series. Neutral fat increased relatively more than the other lipid fractions. These results are in contrast to those of Anderson and Sperry (5) who found a decrease in both absolute and relative amounts of cholesterol ester in pregnancy.

The indications are that the hypertrophying adrenal glands absorb neutral fat from the blood stream first and in greatest quantities. Cholesterol, either free or as the ester, must be absorbed also. The large amounts of neutral fat accumulated may be part of the mechanism for the synthesis of phospholipid by the growing cells in the process of hypertrophy.

TABLE 1

Lipid composition of adrenals in control, pituitary-treated and pregnant rabbits

		CONTROL	PITUITARY TREATED	PREGNANT		PROBABILITIES					
				1-6 days	19-28 days	Control vs. pituitary treated		Control vs. 1-6 day pregnant		Control vs. 19-28 day pregnant	
						t.	p.	t.	p.	t.	p.
Number of animals		20	21	16	9						
Adrenal, mgm. weight	M.*	213	261	281	594	1.65	>0.05	1.95	>0.05	9.96	<0.01
	S.D.	48	58	142	159						
Phospholipid, per cent	M.	3.57	3.57	3.66	2.94	0	>0.05	0.70	>0.05	4.23	<0.01
	S.D.	0.37	0.33	0.40	0.22						
Total cholesterol, per cent	M.	6.30	6.57	5.13	4.67	0.70	>0.05	3.04	<0.01	3.36	<0.01
	S.D.	1.42	1.03	0.66	0.35						
Free cholesterol, per cent	M.	0.86	0.88	0.86	0.96	1.20	>0.05	0.93	>0.05	1.75	>0.05
	S.D.	0.22	0.16	0.22	0.25						
Ester cholesterol, per cent	M.	5.50	5.69	4.27	3.71	0.59	>0.05	3.68	<0.01	4.30	<0.01
	S.D.	1.22	0.83	0.61	0.32						
Neutral fat, per cent	M.	5.82	5.12	8.48	12.54	0.93	>0.05	2.49	<0.05	4.29	<0.01
	S.D.	2.93	1.79	3.47	4.31						
Total lipid, per cent	M.	19.26	18.87	19.50	23.18	0.35	>0.05	0.19	>0.05	2.21	<0.05
	S.D.	4.46	2.44	2.86	4.29						

* M., mean; S.D., standard deviation.

If it be assumed that hypertrophy of glands is caused by increased demand upon them and that the increased size is an indication of increased activity, the increase in the absolute amount of phospholipid in pregnant and insulin-treated rabbits may indicate a relationship between phospholipid content and physiological activity. At least, phospholipid appears to be related to the physiological activity of ovaries and muscles (8, 9).

The absolute amounts of cholesterol and its esters increase in the hypertrophy of pregnancy of rabbits but not of pregnant or parturient

rats (5) nor in insulin-treated rabbits (2). In the hypertrophy produced by cholesterol feeding of rabbits there is an accumulation of cholesterol esters (3). However, in the hypertrophy associated with infections there is a decrease in cholesterol esters (7). It would appear that the cholesterol content of the adrenals might be more closely related to the nutritional status of the animal than to the physiological status of the glands.

The adrenal glands appear to be unique and therefore of unusual interest in their ability to change size and lipid content rapidly under numerous physiological conditions. Most tissues, with the possible exception of the liver, have a constant composition of phospholipid and cholesterol which does not change readily under a wide variety of conditions, including starvation. The adrenal gland should provide an interesting organ for studying chemical composition and physiological activity as measured by hormonal output.

TABLE 2

Analysis of lipid composition of adrenals as milligram per kilogram body weight

		CONTROL	PREGNANT		PROBABILITIES			
			1-6 days	19-28 days	Control vs. 1-6 day pregnant		Control vs. 19-28 day pregnant	
					t.	p.	t.	p.
Body weight, kgm.	M.	2.33	2.59	3.18				
Phospholipid	M.	3.52	3.75	5.46	0.53	>0.05	4.26	<0.01
	S.D.	1.21	1.39	0.92				
Total cholesterol	M.	5.88	5.42	8.67	0.59	>0.05	3.25	<0.01
	S.D.	2.28	2.36	1.77				
Free cholesterol	M.	0.75	0.94	1.82	1.38	>0.05	6.09	<0.01
	S.D.	0.30	0.53	0.66				
Ester cholesterol	M.	5.18	4.48	6.85	1.10	>0.05	2.36	<0.05
	S.D.	1.92	1.88	1.30				
Neutral fat	M.	5.69	9.49	23.77	2.13	<0.05	6.91	<0.01
	S.D.	3.81	6.77	10.42				
Total lipid	M.	18.46	21.29	43.67	0.91	>0.05	6.43	<0.01

SUMMARY

In the hypertrophied adrenal glands of pregnant rabbits there was an increase in the absolute amounts of phospholipid, free and ester cholesterol, neutral fat and total lipid.

The percentage content of phospholipid and cholesterol esters was decreased; free cholesterol and total lipid were not changed; and neutral fat was increased.

A gonadotropic hormone preparation of the pituitary had no significant effect on the adrenal lipids.

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QUANTITATIVE MEASUREMENT OF THE FIBRILLATION THRESHOLDS OF THE MAMMALIAN VENTRICLES WITH OBSERVATIONS ON THE EFFECT OF PROCAINE[1]

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The suspicion has existed for a long time that physiological, physical and chemical influences may create either a state of predisposition or of resistance to fibrillation. Various experimental methods have been designed to test and prove this idea. For these tests different species of animals have been used. Since fibrillation is permanent in the dog's heart, recourse has been taken to a study of apparently related ectopic ventricular rhythms or to comparison of crude fibrillation thresholds in series of treated and untreated animals. For example, Moisset de Espanès (1) found that stronger tetanizing currents were necessary to induce ventricular fibrillation in dogs after administration of 5 to 20 mgm. quinidine or 2 to 10 mgm. fagarina I per kilo. He also noted that the average time for development of spontaneous fibrillation was reduced after occlusion of the ramus descendens in animals previously treated with fagarina I. Thus, in one group of 25 normal dogs, spontaneous fibrillation occurred within 19 minutes in fifteen; in another group of 25 treated with quinidine, 20 fibrillated in less than 16 minutes, and in a third group of 25 treated with faragina I, only 9 fibrillated spontaneously in less than 15 minutes. Whether this means that quinidine acts unfavorably and faragina favorably during coronary occlusion, as this investigator believes, or whether the fibrillation time was fortuitous in these different groups may well be debated. The great variation in the fibrillation time after acute coronary occlusion is well known to experimenters. On the basis of fibrillation times, we could easily assemble three much larger groups from our experimental series of untreated dogs which would show even more significant variations.

Meek et al. (2) used identical dogs by studying the reactions to test doses of epinephrine which in the unanesthetized state only caused "slowing

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with or without escape of the A-V node, bundle or ventricle, but never tachycardia or fibrillation." They found, in 17 dogs, that a similar test dose caused essentially the same effect during light or deep anesthesia with ether or chloroform; but under light cyclopropane anesthesia, 11 of these dogs developed ventricular tachycardia lasting on an average 19 seconds, and one fibrillated. Under deep cyclopropane anesthesia, 16 developed tachycardia lasting 44.5 seconds (average) and one fibrillated. Aside from the two deaths due to fibrillation, the remainder of their proof requires the assumption that development of ectopic ventricular beats or tachycardia is necessarily linked with liability to fibrillate. Such a view would hold if the tachysystolic rather than circus movement concept of fibrillation is favored, which we are not ready to do.

Shen and Simon (3) found that 6 dogs succumbed to "chloroform-adrenalin" fibrillation, whereas of another series of 9 dogs protected by 3 to 5 mgm./kilo procaine hydrochloride, only one fibrillated. Similarly, Shen (4) showed that whereas 8 dogs died from "benzol-adrenalin" fibrillation, in another group of 7 dogs protected by 8 to 10 mgm./kilo procaine, fibrillation never developed. Smaller doses administered to another group of 5 dogs failed to protect. Burstein and Marangoni (5) reported that fibrillation was produced in 3 out of 5 dogs by administering epinephrine during cyclopropane anesthesia; but in 8 dogs previously treated with procaine, only one succumbed to fibrillation. A review of these and similar tests indicates the difficulty of obtaining decisive answers by the use of such bio-assay methods; so many fortuitous factors influence the induction of fibrillation by ischemia or drugs.

The cat has constituted a favored test preparation because the ventricles more generally recover from fibrillation within a minute or so, either spontaneously or by the aid of gentle massage. In this way, repeated thresholds can be obtained on the same animal, before and after various experimental measures. The criteria of changes in sensitivity to fibrillating agents are various and have not been too critically established. They may be grouped, and a few suggestions of their applicability examined.

A first method is based on determinations of the strength or duration (in seconds) of faradic or sine currents which are just sufficient to induce ventricular fibrillation.

Hoff and Nahum (6) applied 60 cycle alternating currents through the limbs of cats, keeping the strength constant but varying the duration. They report that A.C. shocks of shorter duration induce fibrillation following the use of epinephrine, but shocks of longer duration are required after subcutaneous administration of acetylcholine. Moisset de Espanès (1) supplemented his studies by determining the fibrillation threshold of a tetanizing current from an induction coil, and Van Dongen (7), if

we interpret his diction correctly, similarly induced ventricular fibrillation by tetanizing the auricles. We must seriously question the value of such thresholds on several grounds: 1. If, as our results and those of others indicate, a very brief unitary stimulus applied during the vulnerable period of a single systole can induce fibrillation (8, 9), the use of currents extending over numerous beats cannot be expected to be very informative. 2. Employment of prolonged oscillating currents give rather erratic responses (10) even when their form, amplitude and frequency are kept constant and the electrode contacts with the heart are meticulously controlled—details which do not seem to have been taken seriously by many experimenters. 3. Induction shocks of laboratory inductoria are notoriously erratic when examined on cathode-ray screens and maintenance of the same positions of the coils cannot be taken as a reliable index of constancy in faradic shocks.

A second method for determining the resistance of the ventricles to fibrillation consists in determining the duration of fibrillation in cats or the number of times that spontaneous recovery from fibrillation takes place. The first procedure was employed by Smith and Mulder (11). Using 2 seconds' tetanizing current, they noted that the average duration of fibrillation in 6 experiments was reduced from 26.3 to 11.8 seconds during stimulation of the accelerator nerves. Similar effects occurred after injection of epinephrine. However, Ettinger (12) found no relation between strength of current and duration of fibrillation in 40 normal cats; and the same effective current (minimal = 70 M.A.) applied at different times to the same animal induced fibrillation varying greatly in duration. We can confirm this with sine currents applied locally to a small ventricular area. For example, in one cat the duration in 8 consecutive tests ranged from 2 to 92 seconds. The second procedure was used by Van Dongen (7) who concluded that the resistance to fibrillation decreased after denervating the heart or use of various drugs.

A third method consists in comparing the threshold shocks which, applied during diastole, induce a premature contraction. The idea was suggested by one of us to McCord (13) who demonstrated in 1913 that the threshold is reduced by intravenous use of small doses of KCl, and it was again suggested to Mautz (14) who in 1936 demonstrated that topical application of KCl, metycaine or procaine reduces the irritability of the ventricles to single induction shocks. Unfortunately, the test is based on the premises that the induction of fibrillation is entirely a matter of myocardial irritability and that there is a relation between the diastolic stimulus necessary to evoke a premature contraction and the stimulus necessary to fibrillate. This remains to be demonstrated. It must also be clearly understood that if this proves to be a criterion for estimating the resistance to induction of fibrillation, it gives no information regarding the tendency of fibrillation to cease.

A fourth method employed by Beck and Mautz (15) was designed to gain information of such curative properties. It is based on the observation that revival of the dog's heart by use of brief A.C. countershock may not be successful, even after several trials. These investigators report that after procaine has been injected into the right ventricle, such a shock invariably proves successful after previous failure without its use. A large experience has convinced us that success in the use of the countershock method depends on the duration of fibrillation and certain details of technique. We have witnessed instances in which repeated trials failed to defibrillate the ventricles and, when we considered revival hopeless, still another shock was effective. Since this occurred in untreated dogs and since Beck and Mautz originally reported such apparent adjuvant action from application of procaine to the epicardial surface, we believe that their experiences may have been fortuitous.

A fifth procedure, recently suggested by Blumenthal and Tribe-Oppenheimer (16) is based on quantitative determinations of the amount of $BaCl_2$ *which is just sufficient to fibrillate the perfused cat's heart.* The procedure is obviously limited to a single test on one preparation. Using the method, these investigators noted that larger quantities of barium chloride are required after previous use of quinidine.

That a more critical quantitative test is required is indicated not merely by the logical criticisms which can be leveled against the several criteria suggested, but also by the contradictory conclusions which have been drawn from such tests. Thus, chloroform-adrenalin is a dangerous fibrillating combination according to Levy and Lewis (17) and many others (3), but it appears harmless according to the criteria of Meek et al. (2); epinephrine increases the resistance of the heart to fibrillating currents according to Smith and Mulder (11), but reduces it according to Hoff and Nahum (6). Action of the accelerator nerves favors induction of fibrillation according to Rothberger and Winterberg (18), van Dongen (7) and Braun and Samet (19), whereas it reduces the chance of fibrillation according to Smith and Mulder (11). Quinidine, which increases the resistance to fibrillation according to Levine (20), Blumenthal and Tribe-Oppenheimer (16) and many others, seems to make the ventricles more liable to spontaneous fibrillation after coronary occlusion, according to Moisset de Espanès (1), etc.

In 1938, one of us (21) suggested that if, as then seemed possible, a brief single shock causes fibrillation when applied during the vulnerable period of systole, it might be possible to measure the intensity of shocks of known form and duration and so establish a more quantitative *fibrillation threshold*. The likelihood that such a procedure would be applicable and adequate was greatly enhanced after it had been demonstrated (8, 9, 10) that brief shocks or the effective portions of longer stimuli cause fibrillation *only* when they are applied during the vulnerable period and have a mini-

mal effective strength. Since weaker shocks given during the vulnerable period elicit only a single premature beat, it seems probable that a fibrillation threshold so determined measures not merely the irritability of reactive fibers but also any other local state (partial refractoriness, block, altered conduction?) that may be necessary for the initiation of ventricular fibrillation. After it had been further shown that the dog's heart can be promptly and repeatedly revived within 15 to 30 seconds by a modification of the countershock method, successive tests were possible on the same animal. Thus, if it were possible to establish a reasonable constancy of thresholds after repeated revivals of dogs, it might be possible to determine the effects of chemicals, drugs, nerves, or other agencies on the fibrillation threshold.

It is apparent that use of such a quantitative criterion required a searching preliminary study *a*, of the most suitable and easily measurable electric shocks and means for applying them during an occasional vulnerable period, and *b*, of the experimental conditions necessary to keep the fibrillation threshold constant enough to enable recognition of changes induced by various agents. The present communication deals with these problems and analyzes the reactions after procaine, as an example of the magnitude of change which may be expected.

Choice of electrical stimuli and expression of fibrillation thresholds. While previous reports (8, 9, 10) have purposely been limited to qualitative aspects of stimuli and their temporal placement in a cardiac cycle in order to cause fibrillation, we have, during the course of the past year's experimentation, given considerable attention to quantitative possibilities of various stimuli.

It is obvious from data presented in previous papers that confusion can only be avoided by utilizing shocks which are short enough to fall entirely within the vulnerable period of late systole (ca 0.05 sec.). Experience has indicated that, for quantitative work, bipolar is preferable to unipolar stimulation, owing to changing contacts of the beating heart either with a posterior indifferent electrode or with the surrounding tissues, in the event the indifferent electrode is placed elsewhere in the body.

Of the many types of unitary electrical stimuli tested, we have found short rectilinear shocks, 0.01 to 0.03 second in duration, most convenient. Lower voltages can be employed and the danger of current spread is greatly diminished. The durations can be set at the start, but once decided upon, must not be changed during the course of an experiment. In this way, the comparative fibrillation thresholds may be simply expressed in milliamperes, measured from optical records inscribed by a calibrated oscillograph in circuit with the electrodes and stimulator, as previously described.

Methods. The method for generating shocks of different strength and

for applying them successively in opposite directions through nonpolarizable Ag-AgCl electrodes was described in a previous paper (9). However, other procedures could obviously be used quite as well. As in previous work, the shock was applied every sixth beat of the ventricles, in order to allow time for restoration of cardiac irritability and pressure equalization after premature systoles. The shock was advanced or retarded with reference to a cardiac cycle by setting the tempo of the stimulator very slightly out of phase with the heart beat. This was tested by applying very weak shocks (ca 1 M.A.) to the ventricle until a whole series (10 to 15) caused no effect and another similar series all invoked premature contractions. A typical record reproduced as figure 1 shows that the shocks in the three upper records fall progressively later in systole and those of the lower record progressively later in diastole. If sufficiently strong, the shocks in the third record should each fibrillate. Such tests were then repeated with shocks increased in steps of about 4 or 5 M.A. until fibrillation was produced. Figure 2 illustrates graphically the effects obtained on several normal dogs.

Immediately after induction of fibrillation, padded electrodes were applied to the heart and a series of 4 to 7 weak A.C. shocks administered at about 5 second intervals, a process we call serial defibrillation. After a suitable interval (generally 15–20 min.) the test was repeated. It is surprising how accurately and precisely heart rate and blood pressure are generally reëstablished after repeated fibrillations.

Coefficients affecting the constancy of fibrillation thresholds. In view of the fact that the determination of every fibrillation threshold takes approximately 30 minutes and that experiments designed to study the effect of various agents or processes must therefore be prolonged for 5 or 6 hours, it became necessary to determine the constancy of such responses and to avoid all the influences which might possibly affect the threshold. Some of these we have demonstrated to be of importance; others had better be taken into account despite lack of such evidence. Among these coefficients, the following have so far been controlled as far as possible.

1. *Reasonable constancy in temperature of the ventricular surface.* Since the reactivity of tissues is definitely affected by temperature and since de Boer (22) criticized Lewis' observations on ventricular conduction on the ground that the exposed surfaces were allowed to cool, investigators have been worried by temperature changes of the cardiac surfaces, even to the extent of building special protective housings in which the operators' hands could be inserted (23). Incidentally, those who employed such protections did not demonstrate that they actually kept surface temperature the same. For this reason, we determined the surface temperature of the heart by aid of thermopiles in a considerable number of dogs under artificial respiration and with the heart exposed. Variations

up to a maximum of 1.5°C. were found in different regions; but over many hours, any one spot examined changed only a few degrees. Thus, in an experiment of January 9, 1940, a temperature of 32°C. was maintained from 1:50 to 4:29 p.m., after which it fell gradually to 31.1°C. by 5:30 p.m., and, in another experiment on January 10, 1940, the surface temperature of an identical spot decreased progressively from 32.3°C. at

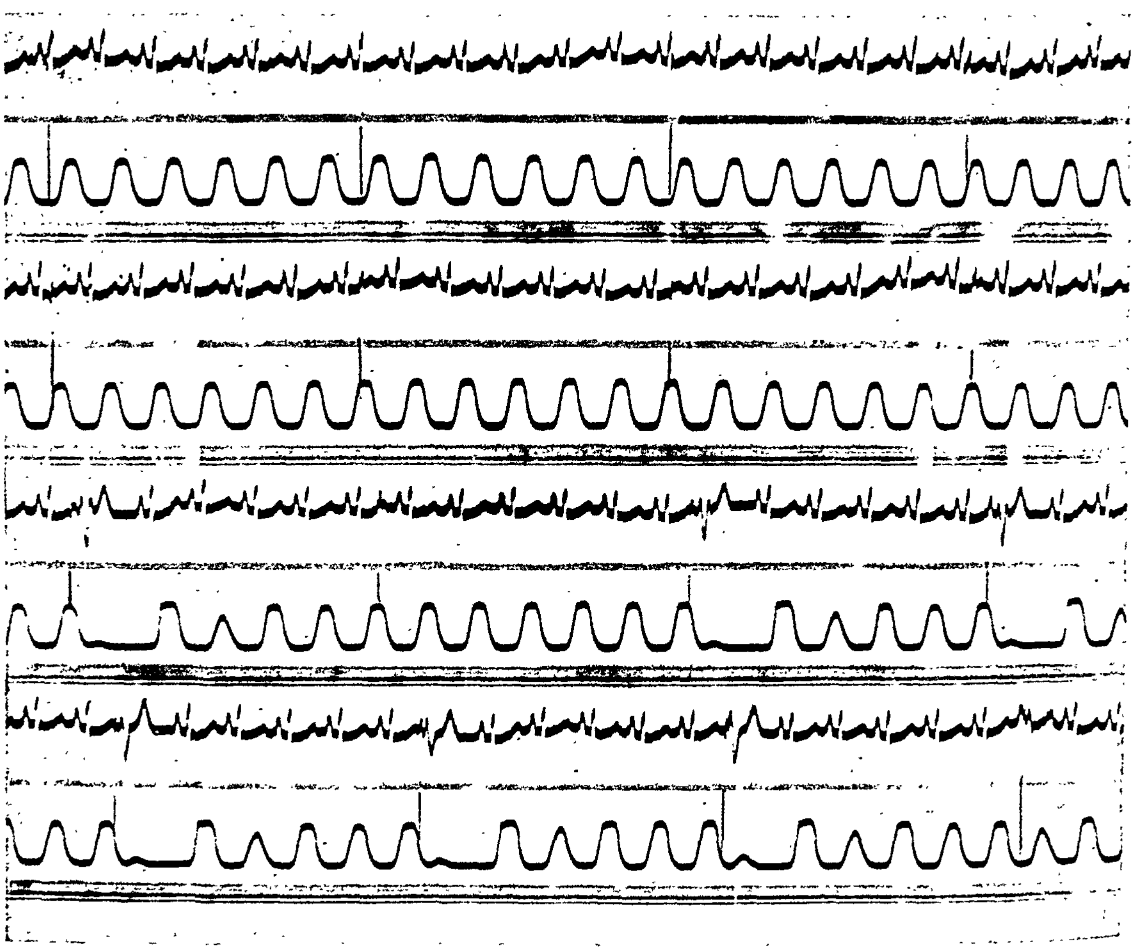

Fig. 1. Upper record E.C.G.; middle, D.C. shock; lower, left ventricular pressure. Note progressive advance in placement of very weak shock with regard to cardiac cycle.

1:05 p.m. to 30.3°C. by 6:10 p.m. We have not been able to discover any changes in threshold with variations in temperature of 3° or 4° as long as the temperature was above 30°C. If such exist, our criterion is not able to detect them.

We have also compared surface and intracardiac temperatures read on a thermometer inserted into the right auricle via a jugular vein and found a

fairly constant difference ranging from 1° to 2° C. in different hearts. When the animal cooled, these temperatures fell together and when heat was applied, they rose proportionately. We were, therefore, led to the conclusion that, owing to the abundant coronary blood supply, the surface temperature of the ventricles is controlled essentially by blood temperature which can be kept fairly constant by control of the heat beneath an animal; it is affected little by radiation, conduction, convection, and evaporation, provided these are not allowed to alter to an extreme degree. Consequently, it is not necessary to utilize elaborate protections to keep the circumambient air constant in experiments of this type. Recording of intracardiac temperatures is a sufficient indication of the trends of surface temperature.

2. *Stimulation of the same point.* The literature contains implications that some regions of the heart may be more responsive to fibrillation than others (24); but such inferences were drawn from use of prolonged tetanizing or direct currents. We have tested the reactivity of various points to D.C. shocks, 0.02 sec. duration, and occasionally found small but not significant differences. Nevertheless, it appears important to stimulate a definite spot in relation to distinguishable anatomical landmarks. The possibility looms large that the route of spread of the initial premature beat over muscle bundles is a factor which is involved in induction of fibrillation. This would naturally differ if different areas were excited. In our experiments, we always selected a region on the left ventricular surface in the angle formed by continuation of the ramus descendens and one of its lower branches spreading to the left. Thus, the chance always existed that the initial spread of the excitation wave would be to the right and left ventricles over the superficial sino-spiral fibers. Repeated stimulation of this area through rigid Ag.-AgCl electrodes so applied that they depress the surface a trifle does not seem to damage the heart even when applied for hours.

3. *Polarization, electrophoresis or anodal-cathodal changes in irritability.* These must naturally be prevented in such prolonged bombardment of a spot by short D.C. shocks. This was accomplished satisfactorily by careful plating of electrodes and meticulous reversal of current in successive shocks through our automatic pole-changer, as attested by conservation of the same recorded value of the stimuli in prolonged tests.

4. *Allowance of an adequate equilibration period after each fibrillation and revival.* Despite the prompt recovery from fibrillation (15–30 sec.), general avoidance of auricular fibrillation and restoration of beats dominated by a sinus pacemaker, the possibility still existed that such influences as temporary asphyxia, brief abolition of intracardiac pressure elevations, residual effects of the countershock, metabolic effects of the

fibrillating process itself, physicochemical changes in the blood during temporary failure of circulation, etc., may so alter the excitability and conductivity of the myocardium that no constancy in fibrillation threshold would be found in repeated trials. In our earlier tests, threshold determinations were made at irregular intervals of 3 to 10 minutes after restoration of normal beats; and we did find significant variations. We then extended the recovery period to 15 to 20 minutes with the hope that such a more extended and constant equilibration period would give better results.

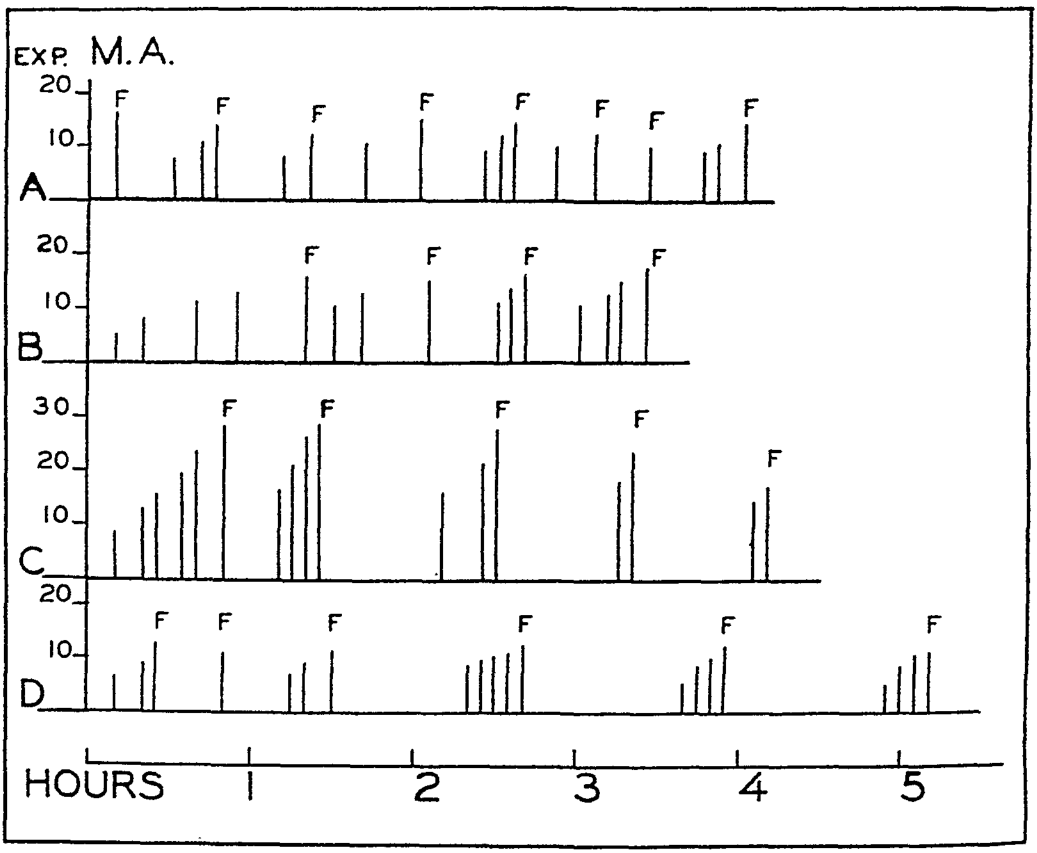

Fig. 2

Doing this we found in the majority of experiments that consistent thresholds were obtainable. Representative experiments extending over 4 or 5 hours are plotted in figure 2. The vertical lines indicate M.A. values of 0.02 to 0.03 second rectilinear shocks used to test the fibrillation threshold; the values represented by lines labeled F causing fibrillation, the others not. Experiment A shows a slight tendency for the threshold to decrease and in experiment C it decreases somewhat more. In experiments B and D, a remarkable degree of constancy obtains. Many such experiments lead to the conclusion that gradual, slight and progressive

variations in fibrillation threshold after use of a drug or operation of a physiological action cannot be taken as a change in resistance to an electric shock; but if the thresholds change abruptly, this seems to be significant.

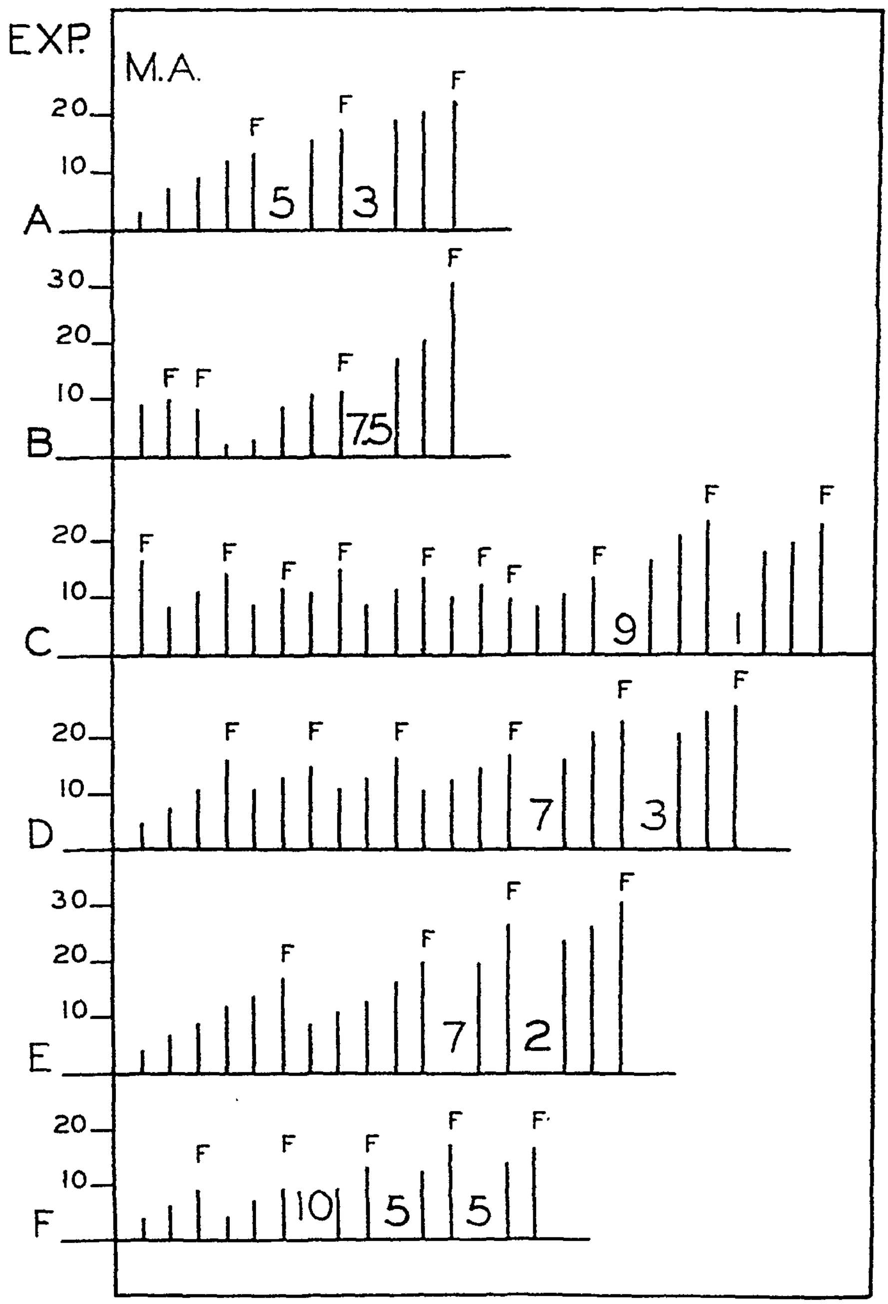

Fig. 3

While we make no claims of having established a threshold of fibrillation which compares with the refinement of stimulation thresholds used in studies of nerve-muscle irritability, we do believe that we have traveled

significantly toward such a goal. When we consider the difficulties of not dealing with a static state of irritability, of requiring introduction of a stimulus at a definite moment of the cardiac cycle, of coping with the significant changes in environment which even a short period of fibrillation may produce, etc., it is not surprising that a greater degree of refinement has not been achieved.

The effect of procaine. That procaine may affect the myocardium was indicated by the experiments of Shookhoff (25) and that epicardial application increases the threshold of induction shocks necessary during diastole for production of premature contractions was demonstrated by Mautz (14). Since the strength of tetanizing currents necessary to produce fibrillation also needed to be increased, he concluded that procaine increases the resistance to fibrillation. Confirmatory evidence as regards the protective action of a simultaneous intravenous dose of 8 to 10 mgm./kilo against benzol-adrenalin fibrillation and of 5 mgm. against chloroform-adrenalin fibrillation was presented respectively by Shen (4) and Shen and Simon (3). Burstein and Marangoni (15) found that 5 mgm. per kilo given previously exerts a protecting action against cyclopropane-epinephrine fibrillation. We have already indicated why we regard these results suggestive but not conclusive.

In 6 dogs, after previous determination of several fibrillation thresholds, 8 to 10 mgm./kilo procaine hydrochloride were injected, usually in divided doses. The results are shown graphically in figure 3 in which the numerals indicate the doses used in milligrams per kilo. In every instance, a significant increase in threshold occurred, the most convincing being experiments B, C, D and E. In experiment E, for instance, a shock 0.02 second in duration fibrillated with a strength of 17 and 20 M.A.; but after slow infusion of 7 mgm. procaine per kilo, 20 M.A. did not fibrillate, but 27 M.A. did. After an additional administration of 2 mgm. per kilo, 24 and 27 M.A. were ineffective but 31 M.A. caused fibrillation. We found no instance in which the heart became entirely refractory to fibrillation and repeated tests showed that such a shock must still be given during the vulnerable period in order to fibrillate; at other times it does not fibrillate. In short, such observations indicate that procaine raises the fibrillation threshold during the vulnerable period as it does the threshold for premature systoles during diastole. It is not a preventive. Revival by countershock occurred promptly, but since this always occurred in normal hearts, no deductions could be drawn regarding the adjuvant action of procaine.

SUMMARY AND CONCLUSIONS

The methods used for determining variations in the sensitivity or resistance of the ventricles to fibrillating agents are briefly reviewed and reasons presented why they are inadequate.

A new quantitative measure for the "fibrillation threshold" of the ventricles is proposed and tested. We believe it takes into account the irritability of non-refractory myocardial fractions during the vulnerable phase of systole, and also any local state that may be necessary for the initiation of ventricular fibrillation. The procedure consists in measuring the current strength of brief D.C. shocks of constant duration (0.01 to 0.03 sec.) which are just able to induce fibrillation when applied during the vulnerable period of late systole to any fixed region of the ventricular surface.

The coefficients which modify the constancy of such thresholds were studied and it was established that, despite repeated fibrillations and defibrillations, the threshold need not vary significantly and does not change abruptly over a period of 4 to 5 hours in untreated dogs. To obtain such relative constancy of fibrillation thresholds, the following conditions were observed: 1. Revival of the heart from fibrillation in less than 30 seconds. 2. Maintenance of the animal's blood temperature within 1 to 2°C. during the course of an experiment, which essentially determines surface temperature as well. 3. Stimulation at the same spot by D.C. shocks applied in alternate directions through non-polarizable electrodes. 4. Allowance of an equilibration period of not less than 15 minutes between one fibrillation and resumption of tests.

The applicability of the quantitative method was tested with reference to procaine which is reputed to increase the resistance of the ventricle to certain fibrillating agents. In six dogs, an increase in threshold, definitely beyond normal bounds was found; after treatment with procaine, a brief shock still fibrillates only when it is applied during the vulnerable period. We conclude that procaine raises the resistance of the ventricles to fibrillation but does not prevent its occurrence.

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THE EFFECTS OF MYOCARDIAL ISCHEMIA ON THE FIBRILLATION THRESHOLD—THE MECHANISM OF SPONTANEOUS VENTRICULAR FIBRILLATION FOLLOWING CORONARY OCCLUSION[1]

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In a previous communication (1) we suggested that the current strength of brief D.C. shocks (ca 0.01 to 0.03 sec.) which are just able to fibrillate the dog's ventricles when applied during the vulnerable period, to a constant small spot of the ventricular surface offers a quantitative measure of the "fibrillation threshold."

The validity of this criterion would be supported and a more general applicability would be suggested if it were found that such a threshold decreases significantly in conditions in which a greater tendency to fibrillation exists. It will probably be generally accepted that this occurs after a large part of a ventricle has been rendered totally ischemic by coronary occlusion.

The present communication presents evidence that the fibrillation threshold is materially reduced after coronary occlusion and suggests a hypothesis which accounts for the spontaneous fibrillation that so frequently supervenes.

Procedure. Dogs weighing 8 to 10 kilos and anesthetized by morphine and sodium barbital were used. The chest was opened under artificial respiration and the heart was suspended in a pericardial cradle. The ramus descendens was dissected for a length of about 1 cm. and prepared for clamping. Left intraventricular pressure, an E.C.G. (lead III), and a quantitative record of the shock through a calibrated G.E. oscillograph were simultaneously recorded. In this way, the strength, duration and moment of application for each stimulus were registered. The stimulus was always applied to a spot which would be in the ischemic territory during coronary occlusion. The duration of the rectilinear D.C shocks (0.01 to 0.02 sec.) and the locus of application were not changed during

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[2] Fellow of the Belgian-American Educational Foundation.

the experiment. The method for creating the stimulus and for advancing it in relation to the heart cycle, as well as the general precautions required to insure constancy in threshold, were described in previous communications (1, 2).

After several tests on the normal heart had established the fibrillation threshold with certainty, the *ramus descendens* was occluded. One or two minutes later, a test for another threshold was started. Such a short interval after occlusion was selected because it has been shown by Tennant and Wiggers (3) that, by this time, the area involved has been affected by the anoxia to the extent that it fails to shorten. Furthermore the ventricles are more easily defibrillated by countershock and the chance for development of spontaneous premature systoles or fibrillation is much less than it is later. Nevertheless, in order to differentiate a possible spontaneous fibrillation from that due to the artificial shock, it was necessary to take records throughout the period of stimulation. Only those fibrillations which were definitely related to a shock (figs. 2–3) were counted as fibrillation thresholds during coronary occlusion.

Immediately after fibrillation, the coronary clamp was released and the ventricles were rhythmically compressed for about 30 seconds in order to revitalize the ischemic tissue by an artificial coronary flow. In these experiments, the aorta was compressed by the second and third fingers, while the left ventricle was pressed rhythmically, thus sending most of the blood expelled into the coronary vessels. Then, a short series of weak A.C. shocks was sent through the ventricles. In general, a greater number was required and the time of revival was extended to 1 or 2 minutes.

After waiting for 20 minutes, a test was repeated while the normal coronary flow persisted. In this way, fibrillation thresholds were determined alternately with and without coronary occlusion.

Results. The general nature of the results in 7 different dogs is graphically charted in figure 1. The abscissae indicate the time scale, and the ordinates denote the strength of the stimuli. The durations of stimuli are given in the legend. Each separate vertical line indicates a strength of stimulus tested. Those resulting in fibrillation when vessels were not occluded are marked N.F.; those inducing fibrillation during occlusion of vessels are designated O.F.

We may profitably analyze experiment 3 in detail. The duration of the D.C. shock was 0.02 second throughout the experiment. In the first determination, a shock of 15 M.A. was required to fibrillate. After defibrillation, recovery, and a 15 minute pause, a threshold shock of 11.5 M.A. caused fibrillation. After still another defibrillation and wait, a third test gave a fibrillation threshold of 12.7 M.A. for the normal heart. After the usual recovery period, the *ramus descendens* was occluded, and 1 minute 30 seconds later, a shock was applied during every sixth normal

beat. The second shock of 5.2 M.A. so applied fortuitously fell during the vulnerable period and fibrillated the ventricle. The possibility of course exists that a still weaker shock might have done so, but it definitely indicates a decrease in threshold. After defibrillation and following another

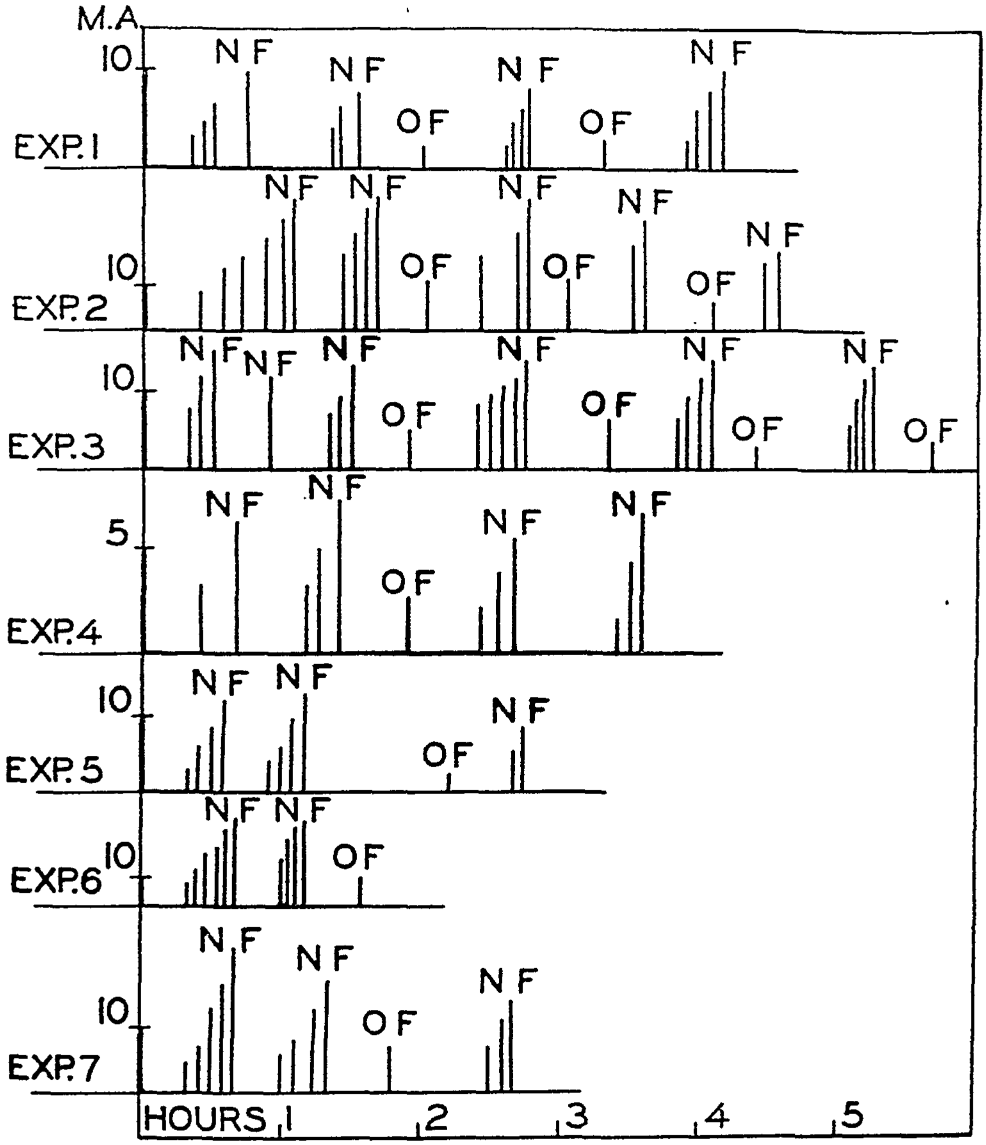

Fig. 1. Diagram indicating the strength of currents causing fibrillation in normal dogs (N.F.) and in the same animals after coronary occlusion (O.F.). Duration of stimuli as follows: Experiment 1—0.01 second; experiment 4—0.016 second; experiments 2, 3, 5, 6, 7—0.02 second.

15 minutes' equilibration period, the ventricle was again stimulated without occluding the coronary artery. As shown in the graph of figure 1, the stimulus value had to be increased progressively to 13.9 M.A. before fibrillation resulted from a shock given during the vulnerable period.

The next test, made during coronary occlusion, showed that a stimulus of 6.4 M.A. induced fibrillation 1 minute 48 seconds after occlusion. Once more, after revival and a recovery period, the fibrillation threshold came back to the same level, i.e., 13.9 M.A. A subsequent test during a new period of occlusion showed that a stimulus of 2.9 M.A. was sufficient to fibrillate the ventricle. After recovery, the normal heart again required 11.5 M.A. to fibrillate, and finally, during a last occlusion, a 3 M.A. shock sufficed to fibrillate 1 minute 52 seconds after occlusion. The repeated alternation of threshold values during and without coronary occlusion leaves no doubt but that ischemia significantly reduces the fibrillation threshold.

Similar reactions were found in the other six dogs, although the experiments were not continued as long. Of supplementary interest are the recoveries of threshold following ischemia, fibrillation and revival. In experiments 1, 3 and 4, the threshold practically returned to that established at the beginning; in experiments 2 and 5 it was decreased somewhat and in experiment 6, no determination could be made after coronary occlusion, since recovery from fibrillation was unsuccessful. Such failure to revive the heart by preliminary massage and serial countershock, particularly after prolonged coronary occlusion, was experienced a number of times. This stresses the greater difficulty of restoring a normal functional state to myocardial fractions subjected to prolonged ischemia.

Critique of interpretations. Tennant and Wiggers (3) demonstrated that the ischemic area no longer shortens one minute after coronary occlusion but expands passively with the rise of intraventricular pressure created by contraction of other myocardial fractions. Since the ischemic myocardium, therefore, does not contribute to the pressure elevation, the question arises whether the ventricular pressure curve still serves as a criterion of the vulnerable period of the region stimulated. The thought is reënforced by observations initially reported by Orias (4) that the contour of the pressure curve is altered and the duration of systole measured therefrom is decidedly shortened. Furthermore, the possibility exists that the refractory periods of fractions in the ischemic area may either be prolonged or abridged.

Records such as are reproduced in figures 2 and 3 not only confirm such suspicions but as a matter of fact give rise to them. It will be noted that the pressure curves are decidedly more peaked than under normal conditions and that the periods of contraction are short. Moreover, a stimulus falling at various moments in relation to the peak induces fibrillation. Thus, while a hasty analysis of figure 2 might properly assign the incidence of the stimulus to the vulnerable phase as previously defined, it also falls rather early in systole; in fact, it coincides with the rise of the T wave of the E.C.G. In figure 3, the fibrillating shock falls definitely on the descending

limb of the pressure curve. The oscillographic tracing shows that fibrillation was induced by a weak shock in all instances.

We must conclude that the span of the vulnerable period in the ischemic myocardium does not bear the normal relationship to the end of systole, demarcated by the pressure curves.[3] Unfortunately, no other record serves as a better guide. The E.C.G. waves, which suggest themselves,

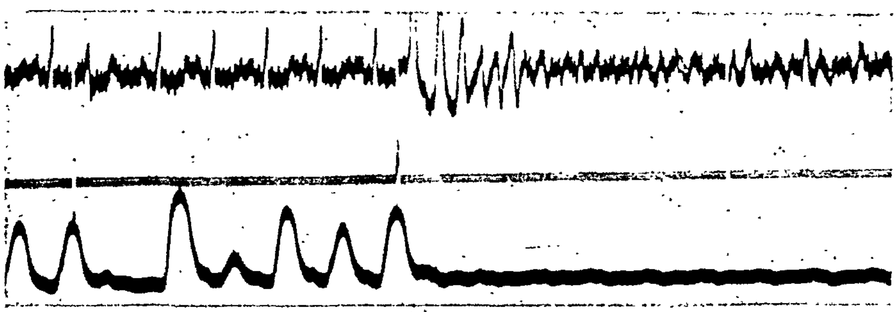

FIG. 2

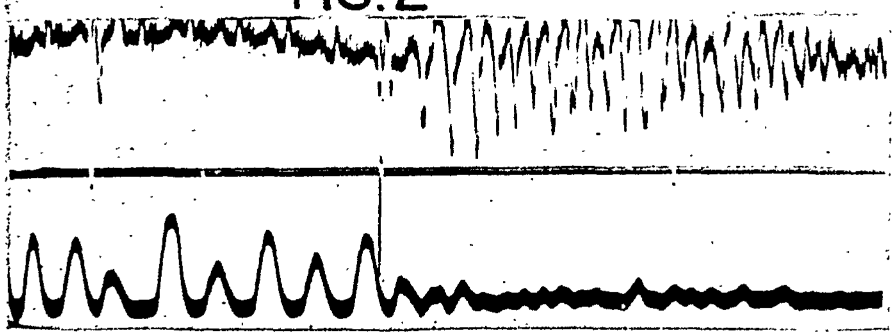

FIG. 3

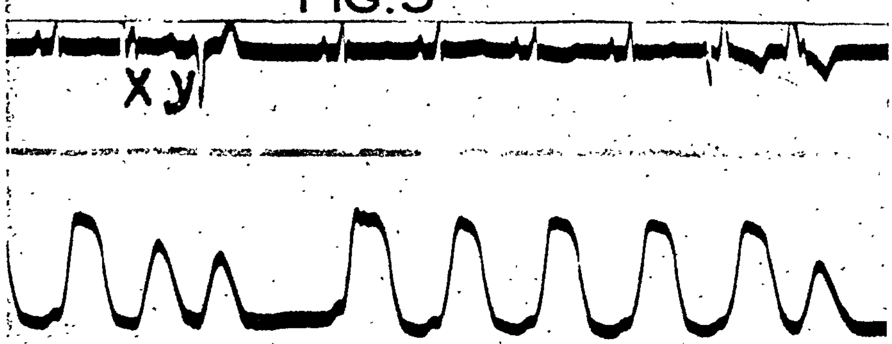

Fig. 4

frequently change as regards the RST segment or T wave and with the abreviation of systole offer no better landmarks. However, it is clear that, even if the vulnerable moments of the ischemic area bear a very fortuitous relation to the pressure curve created by other fractions, it still serves the useful purpose of differentiating fibrillation due to the shock from that

[3] The probability of a similar lack of relation to pressure curves due to ectopic ventricular stimuli has been previously discussed (2).

which may arise spontaneously. Repeated observations indicated clearly that a similar fibrillation is never induced after the pressure wave has returned to its base line. The only difference noted between the reaction of the normal and ischemic myocardium to brief diastolic shocks is the occasional occurrence of two instead of one premature beat; fibrillation never occurs. As illustrated in figure 4, the second of these ectopic beats, Y, may have a different configuration from the first, X, and apparently arises spontaneously from another focus. This, of course, is consonant with the general impression that ischemia enhances the tendency to develop ectopic foci. Apparently it is not even necessary to observe the strict incidence of the shock during the pressure curve, as we have done, in order to compare thresholds of brief shocks which fibrillate before and after coronary occlusion.

Physiological state of the ischemic myocardium. Experimental observations of this part of our investigations have again raised the question as to the physiological state of the potentially infarcted area. In their study of coronary occlusion, Tennant and Wiggers (3) showed that despite a lack of evidence of shortening, the ischemic area remains irritable to induction shocks, and that impulses spread as in normal hearts to both ventricles. Our observations showed that, similarly to normal hearts, an effective brief stimulus induces fibrillation only when applied during the vulnerable period (redefined in relation to the pressure curve), and that the threshold value is significantly reduced. We cannot say whether this signifies solely an enhanced excitability of fractions during the early moments of the non-refractory state or involves in addition a greater asynchronism in passage of myocardial elements out of the refractory state, decrease in refractory period, or differences in conduction which facilitate reëntry.

The observations of Tennant and Wiggers (3) that the ischemic region no longer shortens but stretches, can perhaps not be interpreted as complete failure of the effort of contraction, but rather that the strength of such effort is not sufficient to overcome the stretching force of rising ventricular pressure. In other words, contractions become feeble; they do not stop. The suggestion of Tennant and Wiggers that dissociation of irritability and contractility is demonstrated, must be retracted. In support of these statements we may cite our observations that when the ventricles fibrillate spontaneously or after a brief shock applied to the ischemic area, the whole region participates in fibrillary contractions. Presumably, with removal of the high intraventricular pressures, the contractile processes are able to manifest themselves. Likewise, with application of a series of countershocks, this area comes to rest with the remainder of the myocardium. The reason that revival of vigorous coördinated beats is more difficult following a period of coronary occlusion, is partly that this region remains ineffective in elevating ventricular pressures and partly that so many ectopic beats arise that the heart easily reverts to fibrillation.

The mechanism of "spontaneous" fibrillation after coronary occlusion. We are not aware of the existence of any clear statement with reference to the mechanisms which abruptly initiate fibrillation during coronary occlusion. Descriptions stress the variable periods after which it may occur and vaguely enumerate coefficients which seem to affect the time interval before fibrillation develops. The fact that it is preceded by numerous isolated groups or trains of premature beats is well known. But the question as to their intimate relation to fibrillation and the reasons why they sometimes eventuate in fibrillation and at other times not, is studiously—and perhaps wisely—avoided.

Certain observations and logical conceptions to which they have given rise tempt us to become more venturesome. We have submitted evidence in this and preceding reports that, in order to induce ventricular fibrillation, we must have an effective stimulus, which may be of brief duration and initially operate in a localized region, but which must fall during a vulnerable period. This we interpret to represent an irregular and asynchronous passage of somewhat adjacent fractions from a refractory to a non-refractory state. During spontaneous fibrillation the stimuli obviously arise within the myocardium. Evidence that more than one focus may release them after coronary occlusion is supplied by the frequent occurrence of premature beats, as a prelude. According to our conception, fibrillation would result if one such ectopic impulse fell during the vulnerable period of a normal beat or that of a premature beat induced from another focus. In addition to such proper spacing of impulses, which must be a frequent normal occurrence, it is important that the strength of the stimulus be adequate. This can either be achieved by increasing the intensity of the shock, as in the production of fibrillation in normal hearts by brief, strong stimuli, or by enhancing the myocardial irritability so that a spontaneous impulse acquires an effective fibrillating value. We suggest that this happens in spontaneous fibrillation following coronary occlusion.

According to this hypothesis, acute ischemia introduces dual coefficients. 1. It gives rise to many ectopic foci which release stimuli which are subthreshold as far as induction of fibrillation in the normal myocardium is concerned, and 2, it increases irritability sufficiently so that these stimuli become effective when they fall during the vulnerable phase.

SUMMARY

Alternate determinations of the fibrillation threshold of ventricles with a normal blood supply and during brief coronary occlusion were repeatedly made on the same animal. Rectilinear shocks 0.01 to 0.02 second in duration were applied to an identical area of the left ventricle. The milliampere value of a shock which, when applied during the vulnerable phase, induced fibrillation, served as the quantitative measure of the fibrillation threshold.

Significant reduction in the current strength required to fibrillate was noted during coronary occlusion, e.g., a decrease from 13.9 to 3 M.A. in one experiment. The vulnerable period of the ischemic region does not bear a normal relation to the ventricular pressure curve because the contraction of fibers in the ischemic area does not contribute to the pressure elevation and because the form and duration of the pressure curve are altered. Consequently, stimuli coinciding with the descending limb fibrillate, but those which fall on the horizontal portion of the pressure curve are ineffective in this respect.

The observations of Tennant and Wiggers that the ischemic area no longer shortens but stretches during systole cannot be interpreted as a complete failure of the effort of contraction, but rather that the strength of effort is insufficient to overcome the stretching force of rising intraventricular pressure. When this force is removed during fibrillation, contractile waves are able to manifest themselves again.

Since ischemia reduces the fibrillation theshold to artificial stimuli and also causes formation of ectopic centers, the theory is put forward that spontaneous fibrillation during coronary occlusion is precipitated because such ectopic stimuli now become of threshold value for the hyperirritable myocardium and any one is capable of inducing fibrillation when it falls during either the vulnerable period of a normal beat or that of a premature beat excited from another ectopic center.

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THE EFFECT OF THYROIDECTOMY ON SERUM CHOLESTEROL AND BASAL METABOLIC RATE IN THE RABBIT

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The relationship between the B.M.R. and the level of serum cholesterol in hypothyroidism has been studied by a number of workers. It has been demonstrated in human beings that the B.M.R. rises as the serum cholesterol falls, and vice versa (1). This is best shown by the changes which occur when treatment with thyroid hormone is first begun or when it is discontinued. In children in whom measurement and evaluation of B.M.R. is often difficult, studies of serum cholesterol therefore are of importance as an additional method for diagnosis (2).

It seemed of interest to supplement by animal experimentation our knowledge gained through studies on normal and hypothyroid children. The rabbit was selected as an experimental animal because it has no accessory thyroid tissue and total thyroidectomy can be performed without risk of hypoparathyroidism. It has been shown by Marine and Lenhart (3) that on the fifth to seventh day after thyroidectomy the B.M.R. of the rabbit begins to fall from normal values of 477 to 607 cc. O_2/kgm/hr. and reaches its lowest level of 348 to 355 cc. O_2/kgm/hr. between the 20th and the 30th day after thyroidectomy. Schenk (4) reports similar results.

A rise in blood cholesterol after thyroidectomy has been reported by several investigators (5, 6, 7, 8, 9, 10). The changes reported differ widely, however. Westra and Kunde (8), for instance, report an increase from a concentration of 74 to 94 mgm. per cent in the normal to a postoperative level of 222 to 227 mgm. per cent. This is an average increase of 267 mgm. per cent. Turner, Present and Bidwell (10) find an average increase of only 19 per cent after thyroidectomy. Yoshimura (11) showed that the hypercholesterolemia after the removal of

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the thyroid is only transient. The concentration of the blood cholesterol reached the maximum one to two weeks after operation, and returned to normal in five to seven weeks.

It seemed of interest to follow both the B.M.R. and the serum cholesterol in the same animals. Cholesterol was determined in the serum rather than in the whole blood as changes are more marked in the serum or plasma than in the whole blood.

MATERIALS AND METHODS. Rabbits of an inbred strain of Dutch and American Blues were used. All except one (no. 31) were males. They were kept in separate metabolism cages and fed on a stock diet described by Hyde (12). (Formula of diet: coarsely ground wheat 5000 grams, chopped alfalfa 5000 grams, flaxseed meal 250 grams, calcium carbonate 85 grams, sodium chloride 55 grams.)

Thyroidectomy was performed under ether anesthesia. At the conclusion of the experiments the absence of thyroid tissue was verified by autopsy.

The B.M.R. was measured with the simple apparatus for metabolic measurements described by Tainter and Rytand (13). The apparatus was built on a larger scale than the original so that a rabbit could be conveniently placed in the chamber and changes in volume from 10 to 250 cc. could be recorded. The chamber was large enough to permit air to be used instead of oxygen. The data obtained with this apparatus compared well with those of Marine and Lenhart (3), who used a Haldane apparatus.

Blood was taken from the ear vein and the total cholesterol was determined in the serum by the method of Bloor adapted to the Evelyn colorimeter (14).

Serum cholesterol and B.M.R. after thyroidectomy. Observations were made simultaneously on the serum cholesterol and B.M.R. of 15 rabbits over periods of 16 to 19 weeks following total thyroidectomy. Prior to operation the values for the normal were determined and in many instances repeated observations were made.

During the first six weeks after operation there was a gradual decrease in B.M.R. to about 40 per cent below the pre-operative level. After thyroidectomy the serum cholesterol showed a sharp initial rise varying from 81 to 340 per cent (average 171 per cent) above the pre-operative level. After the first rise the serum cholesterol fluctuated markedly, finally becoming stabilized after about 12 weeks at a value 14 per cent to 221 per cent (average 80 per cent) above the pre-operative level. On figure 1 all single determinations are recorded as dots. This figure illustrates the great variations of serum cholesterol after thyroidectomy in contrast to the gradual decrease in B.M.R. On figure 2 three representative experiments of this series are shown.

Repeated determinations showed that the serum cholesterol never fluctuated more than 36 mgm. per cent in any rabbit before operation. This corresponds closely to the observation of Horiuchi (15), who reported values of plasma cholesterol of 40 to 100 mgm. per cent in normal rabbits, with individual fluctuations less than 30 per cent. The instability of the serum cholesterol following thyroidectomy was in marked contrast to this. During the first 12 weeks after operation we found fluctuations as great as 224 mgm. per cent in some rabbits. After the 12th week there was apparently less tendency for the cholesterol to

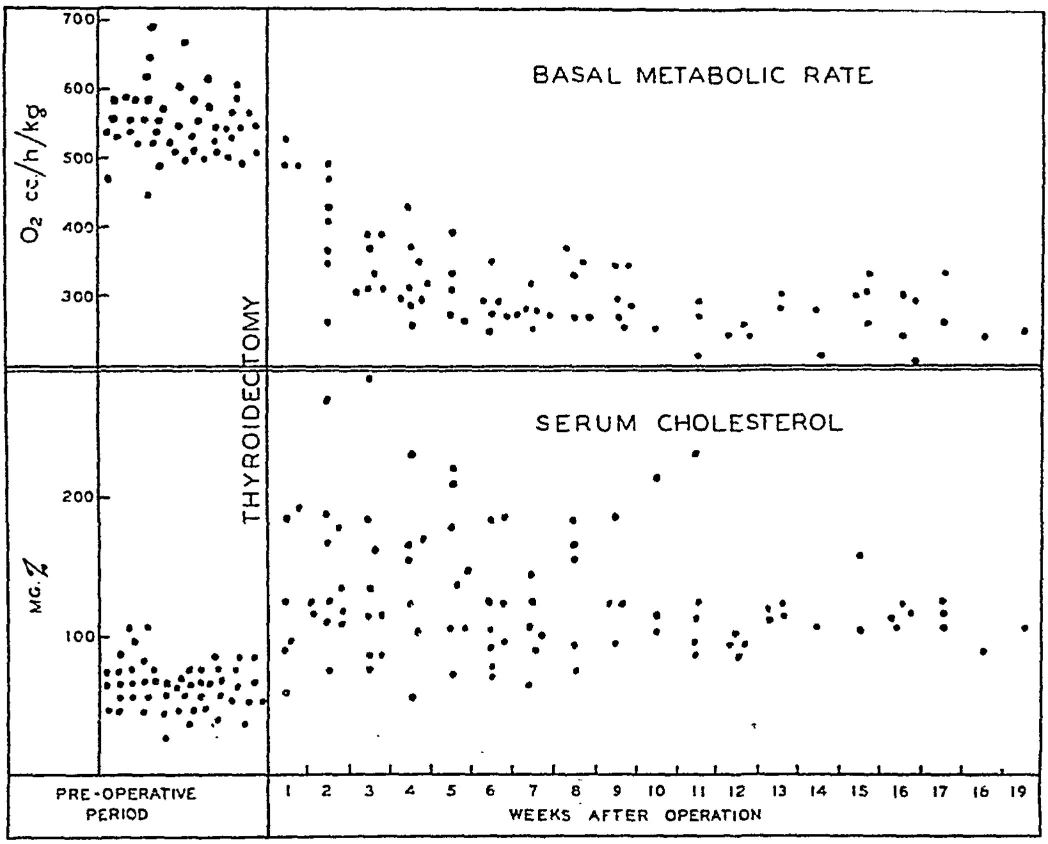

Fig. 1

fluctuate since the greatest variation observed was 55 mgm. per cent. However, the number of observations in later weeks was too small to warrant definite conclusions.

The instability of the serum cholesterol after thyroidectomy suggests that the thyroid is an important factor in regulating blood cholesterol. The marked fluctuations which occur immediately after operation could be explained by a removal of the regulatory mechanism. It is conceivable that after several weeks a new regulatory mechanism is established in which the thyroid is no factor. The fact that there is a gradual and

regular decrease of the B.M.R., whereas there are wide fluctuations of serum cholesterol after thyroidectomy, indicates that there is no direct relationship between B.M.R. and blood cholesterol, although both are influenced by the thyroid. That changes in the blood cholesterol level are not directly related to the metabolic rate has been shown previously in human beings by the fact that dinitrophenol raises the B.M.R. without causing a drop in serum cholesterol (16, 17).

The sensitivity of normal and thyroidectomized rabbits to the administration of thyroxin. It has been shown by P. E. Smith and his associates (18) that the effect of thyroid extract on the metabolism of thyroidec-

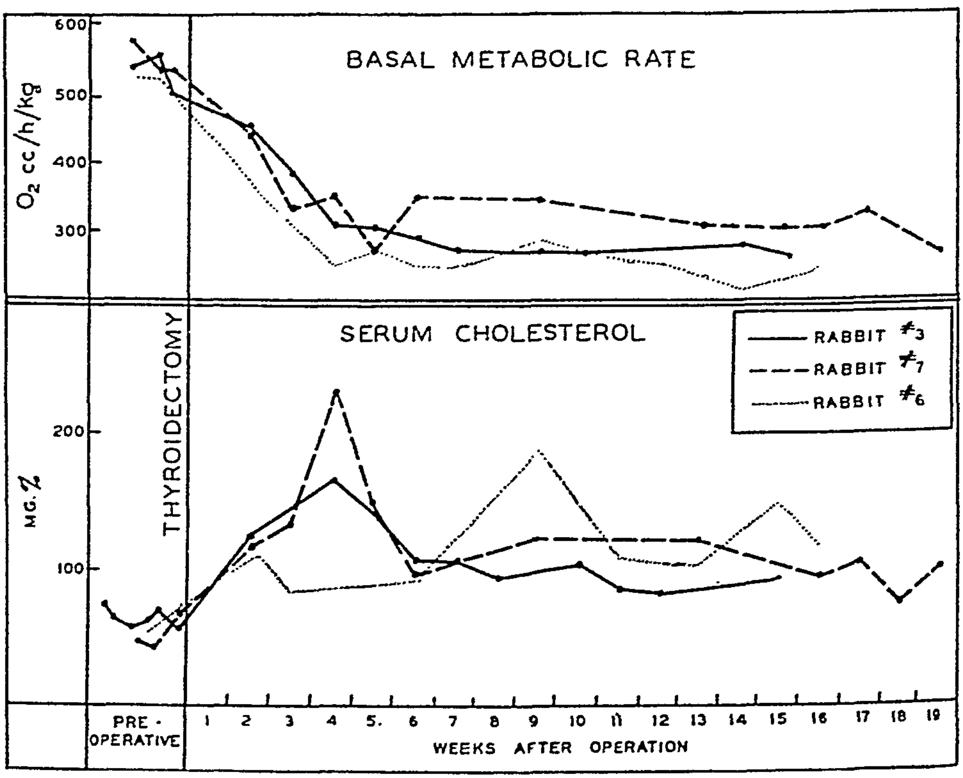

Fig. 2

tomized rats is much greater than on that of normals. Similar observations have been reported in dogs (19, 20). In human beings a single dose of thyroxin causes a greater effect in untreated hypothyroid patients than in treated patients or in normal individuals; and, in addition, the effect lasts over a considerably longer time (21, 22).

Our experiments on rabbits showed the same contrast between the normal and the hypothyroid animal. Thyroxin (Roche-Organon) was injected subcutaneously after a base line had been established. These experiments are summarized in table 1. The basal metabolic rate was used as an index of thyroid effect rather than the serum cholesterol

level because of the great fluctuations in serum cholesterol which occur in hypothyroid rabbits. The data in table 1 indicate that the effect of thyroxin is much greater in hypothyroid than in normal rabbits. It is further to be seen from the last column of this table that the lethal

TABLE 1

Sensitivity of normal and thyroidectomized rabbits to the administration of thyroxin

RABBIT NUMBER	DOSE OF THYROXIN	MAXIMUM RISE OF B.M.R.	DAYS AFTER WHICH MAXIMUM IS REACHED	REVERTED TO NORMAL AFTER	MAXIMUM DECREASE IN CHOLESTEROL	
Normal rabbits						
	mgm.	*per cent*		*days*	*mgm. per cent*	
3	0.5	9	2	3	10	
16	0.8	13	2	3	0	
6	2.0	11	4	9	0	
1	3.0	17	3	6	32	
3	5.0	27	2	7	15	
7	5.0	35	3	8	2	
5	5.0	25	2	4	8	
8	5.0	28	3	5		
2	5.0	44	2	8	12	
9	5.0	31	4	6		
8	7.0	6	3	4		
43	10.0	69	3	6	32	
42	10.0	38			14	Died on 2nd day
43	20.0	79	3	5	28	
Thyroidectomized rabbits						
14	0.5	32	2	4	53	
13	0.5	89	4		21	Died on 7th day
14	0.8	44	3	8	38	
6	1.0	92	8		30	Died on 13th day
10	1.0	59			56	Died on 2nd day
22	2.0	122	5	9		
21	5.0	174	4	10	56	
3	5.0	65			57	Died on 2nd day
21	5.0	93	6		26	Died on 8th day
26	10.0	78			91	Died on 4th day
27	10.0	47			30	Died on 3rd day
31	10.0	96	4	9	36	Died on 15th day

dose of thyroxin for the hypothyroid rabbit may be smaller than that for the normal. All normal animals survived except one (no. 42) injected with a single dose of 10 mgm. In the hypothyroid group doses as low as 0.5 mgm. sometimes proved fatal. One thyroidectomized rabbit (no. 31) died 6 days after the B.M.R. and serum cholesterol had

reverted to normal. Death was probably due to thyroxin poisoning, as no other cause could be discovered at autopsy.

A marked increase in creatinuria is caused in hypothyroid children by a single dose of thyroxin (22). Creatine studies were unsatisfactory in most rabbits as the creatine excretion showed marked fluctuations. In a few rabbits having fairly constant creatine excretion it was found that small doses of thyroxin (0.5 mgm.) caused increased creatinuria in thyroidectomized animals, whereas large doses (5.0 mgm.) were required to cause any effect in normal animals. For instance, in a thyroidectomized rabbit (no. 14) a sharp rise in creatine excretion (48 hr. period) from a level of 8 mgm. to a level of 48 mgm. following injection of 0.8 mgm. of thyroxin was observed, whereas the creatine excretion of the control animal (no. 16) was not affected, staying on its level of 15 mgm. creatine excreted (48 hr. period).

Discussion. It is generally recognized that there are marked differences in the cholesterol metabolism of human beings and rabbits. In spite of this fact, the studies reported here show similarities to certain observations made by the writers (2, 22, 23) upon hypothyroid children.

1. In a group of 21 untreated hypothyroid children the serum cholesterol varied from 145 to 660 mgm. per cent compared to values ranging from 98 to 308 mgm. per cent in normal children. In the hypothyroid rabbits the range was from 70 to 290 mgm. per cent compared to from 33 to 107 mgm. per cent in normal controls. In both human beings and rabbits the ranges of the hypothyroid and normal groups overlap.

2. In following the cholesterol level in given individuals over long periods, greater spontaneous fluctuations were encountered in hypothyroid than in normal children. The fluctuations in hypothyroid patients were as great as 200 mgm. per cent, whereas in normal children fluctuations never exceeded 83 mgm. per cent and generally were much smaller. In thyroidectomized rabbits individual fluctuations were as great as 224 mgm. per cent, whilst in the normal the greatest fluctuation found was 36 mgm. per cent. The marked instability of the serum cholesterol in hypothyroid individuals may account for the low values which are frequently observed in hypothyroidism.

3. The sharp rise in serum cholesterol after thyroidectomy in the rabbit is similar to the rise observed in hypothyroid children after withdrawal of thyroid medication. In normal children withdrawal of thyroid medication has no such effect. In some of the hypothyroid children serum cholesterol rose to a peak after withdrawal of medication, and then dropped to a lower level (23). This rise and fall is very similar to that observed in rabbits after thyroidectomy. It is quite possible that such peaks in the concentration of cholesterol in the serum might have been observed more frequently in hypothyroid children if thyroid

treatment had been withheld for a longer period of study. This, however, did not seem justifiable from a therapeutic viewpoint.

4. The greater sensitivity of hypothyroid rabbits in comparison to normals to a single injection of thyroxin, as evidenced by a greater rise in B.M.R., drop in serum cholesterol, and in some cases increased creatine excretion, is very similar to the greater sensitivity observed in hypothyroid children (22).

Although both the studies on rabbits and those on children produce strong evidence of the relationship between the thyroid gland and cholesterol metabolism, they shed no light on the mechanism of the action of the thyroid hormone on the serum cholesterol level.

SUMMARY

Thyroidectomy in the rabbit is followed by a sharp rise in serum cholesterol varying from 81 to 340 per cent (average 171 per cent) above the preoperative level. After the first rise the serum cholesterol fluctuates markedly, finally becoming stabilized after about 12 weeks at a value 14 to 221 per cent (average 80 per cent) above the base level. The basal metabolic rate does not show marked fluctuations, but decreases gradually in six weeks to about 40 per cent below the preoperative level. Thyroidectomized rabbits are more sensitive than normals to a single injection of thyroxin, as can be shown by following the changes in the basal metabolic rate, serum cholesterol and creatine excretion. The relations of these findings in rabbits to similar studies in human beings are discussed.

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WATER DISTRIBUTION AND SEXUAL SKIN OF THE BABOON

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The sexual skin of the perineal region of many primates undergoes rhythmic alterations in size as well as in color throughout successive menstrual cycles. This phenomenon has been studied by several workers, especially by Zuckerman (1937) who has summarized the evidence in support of the theory that the post-menstrual turgescence is due to the increased secretion of estrogens, and that their sudden post-ovulatory suppression is responsible for the rapid deturgescence. Gillman (1935) reported a long series of observations on the chacma baboon, *Papio porcarius*, questioning Zuckerman's early (1930) statement that there is a regular 28-day cycle, but showing that the cycle is somewhat irregular (29 to 63 days) the average of 20 cycles being 42 days. He also attempted a quantitative description of the changes in the sexual skin by recording four linear measurements by which the rate and extent of the changes may be evaluated. On the basis of later experiments, Gillman (1938) concluded that other factors in addition to estrone withdrawal are responsible for deturgescence and bleeding. Further evidence for the participation of sex hormones in water metabolism was given in a note by Zuckerman, Palmer and Bourne (1939) who point out that estradiol has a specific effect in promoting the movement of water in the rat from muscle and certain viscera to the uterus, vagina and skin. The present paper adds new quantitative observations on the sexual skin and the movements of body water.

MATERIALS AND METHODS. The principal subject of the following report is a female *Papio hamadryas*, the "sacred" baboon, which came to this laboratory in May, 1934. In June, 1934, craniotomy was performed and a small incision was made in the corpus callosum, as a control operation for other studies then in progress. Recovery was prompt and uneventful, and no lasting effects of any kind can be attributed to that procedure.

During periods of continuous observation the animal was kept indoors in a small cage arranged for urine collection. The food supply was varied

[1] Aided by a grant from the Fluid Research Funds of the Yale University School of Medicine.

and adequate. Water was usually available, but the animal often filled the water cup with feces. When water intake was to be accurately measured the animal was permitted to drink from a cup held by the observer, water being offered in this manner at least twice a day. The volume of the sexual skin was measured by an immersion method. The baboon, which was quite docile and moderately tame, was held vertically with the hind legs somewhat elevated so that the dependent sexual skin could be dipped into a large vessel of water until it was completely submerged. It was easy to effect complete immersion of the sexual skin with practically none of the rump of the animal. The vessel was supported on the platform of a large springless balance, and the rise in weight was the measure of the volume of water displaced. The error did not exceed 0.2 liter. The weight of the sexual skin was calculated by assuming a specific gravity of 1.05.

The volume of extracellular fluid was estimated as the volume of distribution of injected thiocyanate (Lavietes et al., 1936). A visual colorimeter was used with 1 ml. cups and a Wratten C2 (blue) filter. The amount of thiocyanate bound in the plasma of the baboon was not studied and no correction for possible binding has been applied. Mixing was rapid, as shown by the identity of the thiocyanate concentrations in serum taken at various intervals between 40 and 160 minutes after injection. Sulfanilamide was tried as a means of measuring total body water (Marshall et al., 1937) using the improved method of assay of Marshall and Litchfield (1938), but it was found that the baboon, like man, rapidly conjugates the substance, the combined form appearing both in the urine and in the blood. For that reason the significance of the calculated volume of distribution is of questionable significance and the method was discarded. In its place urea was used, the analytical method being that of Peters and Van Slyke (1932, p. 373). Blood samples were drawn 30, 60 and 90 minutes after the intravenous injection of about 4 grams of urea, and the calculation was based on the blood urea increment extrapolated to the time of injection. Plasma volume was measured with the blue dye, T-1824, using the Evelyn colorimeter with filter 600. Hematocrit determinations were made in Wintrobe tubes using the first blood sample of each experiment. Plasma water content was found by drying 1 ml. at 105°C.

Observations and experiments. The period of most careful observation extended from June 7, 1937, when menstrual bleeding occurred, to July 9, 1938, when it again appeared, making the end of eleven cycles of swelling and shrinking of the sexual skin. In a few instances actual bleeding was not recorded, the cycles being determined by the changes in the sexual skin. The exact length of each period is therefore unknown; the average of the eleven periods is 36 days, which is well within the range

noted by Zuckerman and by Gillman. Since measurements of sexual skin volume were not made in every cycle it cannot be said that the maxi-

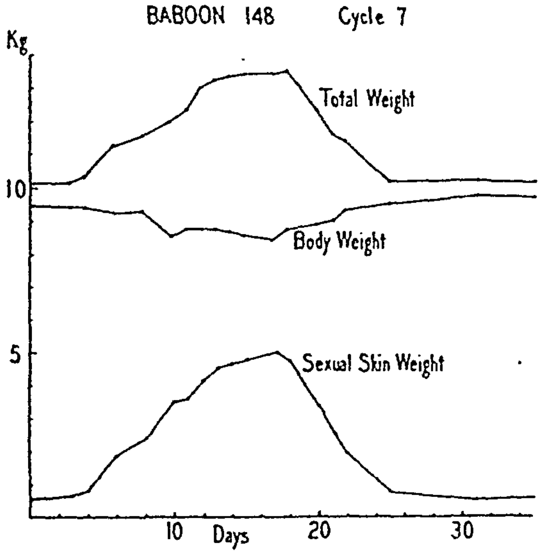

Fig. 1

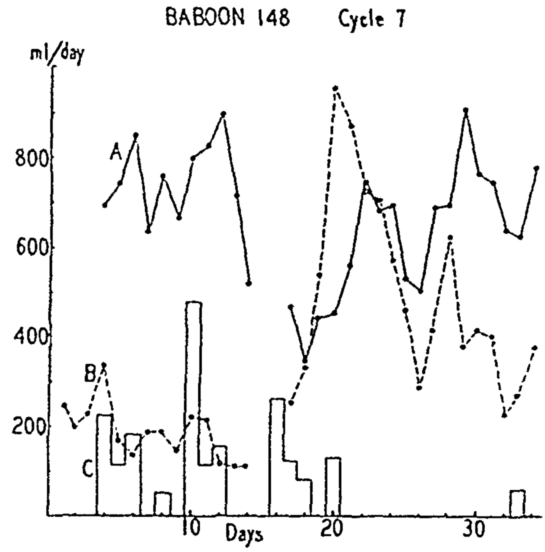

Fig. 2

Fig. 1. Menstrual bleeding on days 1–3 and 35. Nembutal anesthesia on day 15, for measurement of extracellular fluid volume.

Fig. 2. A. Total water intake. B. Urine output. C. Water drunk.

TABLE 1

	ANIMAL 148			ANIMAL 842	
	Weight, kgm.				
	10.4	11.1	12.2	10.0	11.2
Sexual skin:					
Relative swelling, per cent	0	50	100	0	100
Per cent of total weight	2	20	35	2	16
Hematocrit: per cent cells	42	44	47	42	50
Plasma water, mgm./ml	945	941	949	933	935
Thiocyanate vol., per cent of total weight	27	30	42	27	32
Urea volume, per cent of total weight	60		66		

It was not possible to make all the desired measurements simultaneously, and so the data in table 1 have been assembled from different cycles to illustrate the typical conditions at certain particular stages of the cycle. The second animal, no. 842, was a Guinea baboon, *P. papio*, which did not exhibit so great a swelling of the sexual skin as is seen in *P. hamadryas*.

mal swelling is always the same, but volumes of 3.6, 5.0 and 4.4 liters in cycles 6, 7 and 9 respectively suggest that the maximum is variable.

The time course of the turgescence and deturgescence of the sexual skin was studied by almost daily measurements during cycles 6 and 7, at which

time the major factors of water balance were also recorded. The data for cycle 6 were tabulated in the preliminary report (Clarke, 1938), while those for cycle 7 are summarized in figures 1 and 2. The total water intake includes, with the water drunk, the free water in the food and the calculated water of oxidation, both of the latter being approximated from available data of food composition. The urine output is not corrected for possible losses by evaporation or incomplete cage drainage. In figure 2 the same ordinate scale is used for total weight and computed sexual skin weight. The differences between the two are plotted as the "body" weight in order to emphasize the shift of material from the body to the sexual skin.

Discussion and conclusions. The menstrual cycle, characteristic of the primates, is accompanied in some of them by periodic changes in sexual skin and in other parts of the body. In animals having protruding sexual skin the changes vary tremendously in extent and reach their maximum in some members of the baboon tribe. In the pigtailed macaque, *M. nemestrina*, studied by Krohn and Zuckerman (1937), an increase of 18 per cent in total weight of the animal was found during the sexual swelling, and it was implied that the weight change was confined to the sexual skin. That the situation is not so simple in the baboon is indicated by the results given in the preceding section. The shift of weight to the sexual skin is controlled by so powerful a stimulus that the remainder of the animal loses weight to the extent of at least 1.2 kgm. (e.g., cycles 6 and 7). The blood can be at best a minor contributor. The total blood volume was found to lie between 800 and 900 ml. in a few measurements with T-1824 and the hematocrit. Any alterations in plasma volume at different stages of swelling are not sufficiently great to be established by the data available. The rise in cell fraction (hematocrit) as the swelling proceeds may be a simple replacement of menstrual loss, although transfer of plasma to an extravascular site would give the same result. Since the water content of the plasma, which was accurately measured, was constant, such a transfer would have to be whole plasma including the plasma proteins.

The state of water in the swollen sexual skin is of considerable interest, and a number of observations bearing on the point were made. That it is not a simple edema is shown by 1, the texture and lack of surface wetness on cutting out a biopsy specimen for histological examination, and 2, by the failure to obtain any exudate from a perforated 18-gauge needle deeply inserted for two hours during anesthesia. The source of the mucoid material is unknown, but if it derives directly from the blood there must be such a local alteration in capillary permeability that the colloidal dye, used for plasma volume determination might also escape from the blood vessels. Two biopsy specimens were taken immediately

after plasma volume determinations (sexual skin volumes 0.9 and 2.5 liters) but no blue coloration was visible to the eye, although enough dye had been given to be clearly visible in samples of plasma. It is still held, however, that until this question is answered little weight can be given to plasma volumes thus measured.

Definite conclusions regarding the body water may be drawn from the data on total and extracellular water (volumes of distribution of urea and thiocyanate). The distribution of total weight between body and sexual skin is based on accurate measurements. A slight fall in the extracellular water fraction in the body when the sexual skin is swollen is based on the indication by hematocrit of hemoconcentration, which in the present situation should more or less parallel the extracellular fluid of the body. The animal's relatively increased thirst at this time is further evidence of body dehydration. The intracellular water, calculated by subtracting the thiocyanate volume from the urea volume, is about 3.4 liters when the sexual skin is shrunken, and at the height of the turgescence it has been reduced to about 3.0 liters. This is further proof that the sexual skin swelling is a primary expansion of the extracellular compartment in the specialized tissue, at the expense of cell water and of the interstitial water of the body.

During the phase of swelling the water intake from all sources naturally exceeds the urine output several fold, but promptly at the start of shrinking the urine output is greatly augmented and appetite for fluid water disappears. In cycle 7 the phase of rapid shrinkage occupied days 18 to 24 inclusive, during which time the total water input was 3.84 liters (of which only 0.2 liter was drunk as fluid) while the output by the renal route alone was 4.73 liters. For eleven consecutive days the animal did not drink, and during the 17 days from the start of shrinking to the next menstruation only 0.25 liter of liquid water was imbibed.

When the weight of the sexual skin is plotted against the weight of the body no evidence is found that the swelling and shrinking phases pursue different courses. The data for cycles 6 and 7, thus plotted are scattered fairly closely to a straight line with slope $= -0.2$, which indicates that each kilogram change of sexual skin is accompanied by a 0.2 kgm. change of the body in the opposite direction.

SUMMARY

The sexual skin of the baboon achieves its state of turgescence with little if any change in its cellular volume but a large increase in its interstitial volume. The increase in weight of the organ is in part (20 per cent) contributed by the animal's own body, and the remainder (80 per cent) by the addition of new material from the outside. Thirst and renal func-

tion are closely coördinated with the changes in water balance, in that the animal exhibits thirst and relative oliguria during the rise of the swelling, and the reverse while resorption is going on.

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THE EFFECTS OF DECREASED BLOOD OXYGEN AND INCREASED BLOOD CARBON DIOXIDE ON THE FLOW AND COMPOSITION OF CERVICAL AND CARDIAC LYMPH[1]

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Though the effects of the inhalation of air deficient in oxygen or high in carbon dixoide have been studied in connection with the physiology of many bodily functions, very little has been done with regard to the effects of these agents on the flow and composition of lymph. Landis (1927–1928), by means of his micro-injection technique, studied the effect of complete lack of oxygen on the permeability of the capillary wall to fluid and to the plasma proteins, and was able to show that complete lack of oxygen for 3 minutes brought about a four-fold increase in the rate of capillary filtration and at the same time allowed the plasma proteins to escape into the surrounding tissue spaces to such an extent that the effective osmotic pressure of the plasma was reduced to one-half of the normal value. Landis also reported that complete saturation with carbon dioxide of the fluid bathing the particular tissue with which he was working caused only a slight increase in the rate of capillary filtration and allowed the escape of none of the plasma proteins, while half saturation of the bathing fluid with carbon dioxide caused no change at all in the rate of formation of capillary filtrate.

Gesell (1928) reported that low oxygen and high carbon dioxide increased the flow of thoracic duct lymph, with a subsequent retardation during recovery. He stated, also, that as the flow increased the lymph became increasingly turbid, and suggested that this turbidity indicated increased permeability of the vascular membranes involved. He did not state, however, the effective concentrations of these agents. Though not directly related to the problem of lymph production, the studies of Campbell (1929), who compared the pathological effects of prolonged exposure to carbon monoxide and to very low oxygen tensions, suggest the possible effects of these agents on the production of capillary filtrate and subsequently of lymph. As a result of his studies, he reported that prolonged exposure to carbon monoxide and to low oxygen tensions caused among

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other things the production of edema and dropsy, undoubtedly the result of increased capillary filtration, which would favor increased lymph production.

McMichael and Morris (1936) attempted to determine the effect of acute oxygen lack on capillary permeability in man by making measurements of arm volume, following the method of Smirk (1935–1936). They observed that the inhalation of gas mixtures containing concentrations of oxygen as low as 9.5 per cent was without effect on the rate of swelling of the human arm, and that high percentages of carbon dioxide in the inspired air were also without effect on the rate of swelling, even when combined with low oxygen percentages. Unfortunately, the duration of these experiments was not stated. Finally, Saslow (1938) reported that solutions of 3 per cent acacia and 19 to 24 per cent ox red cells in Ringer's solution could be perfused through frogs for periods up to 6 hours without the appearance of microscopically detectable edema in the web. He stated that the effectiveness of this solution in preventing edema formation appeared to be due to its high content of available oxygen.

The present work consists of the results of twenty experiments performed on dogs in an attempt to determine the effects of the inspiration of gas mixtures low in oxygen and of underventilation upon the production and composition of cervical and cardiac lymph.

Experimental technique. All of the experiments were performed on healthy young adult dogs under nembutal anesthesia (40 mgm. per kgm. intravenously). The procedure was essentially that described by McCarrell (1939) for the continuous collection of cervical lymph, this preparation being known by us as the "nodding dog."

While the preparation was in progress, an infusion of 20 cc. per kgm. of warm Ringer's solution was given to ensure thorough hydration of the animal. Arterial blood pressure was recorded by the usual mercury manometer. The animal's temperature was measured at various intervals by means of a rectal thermometer.

Except when the animal was made to breathe room air, gas mixtures of known concentration were made up in pressure cylinders and delivered to an 80-litre spirometer, from which the gas was drawn into a respiration pump designed so that any given volume of air could be delivered into the lungs at the rate of 14 inspirations per minute. Rebreathing experiments were performed by establishing a closed circulation between the spirometer and the animal, soda-lime being used for the removal of carbon dioxide. In this manner the oxygen saturation of the blood could easily be determined at the exact time that the lymph flow began to change.

Arterial blood samples were collected from the femoral artery into blood tubes in the usual manner, and were analyzed in the Van Slyke gas analyzer for oxygen and carbon dioxide. Gas samples from the spirometer were analyzed in the Haldane gas analyzer.

At the beginning of each experiment 1 cc. of 1 per cent curare diluted to 20 cc. with Ringer's solution was injected intravenously, in order to eliminate the forced inspiratory movements which always occur with the inspiration of low oxygen or high carbon dioxide mixtures. Such forced inspirations, if allowed to persist, would produce wide irregularities in the flow of lymph. Both the nembutal and the curare were repeated during the experiment in small doses as required.

RESULTS. *Exposure to low oxygen mixtures of known concentration.* In order first to determine the effect of low oxygen pressures upon the flow of cervical lymph, the animals used in the first few experiments were

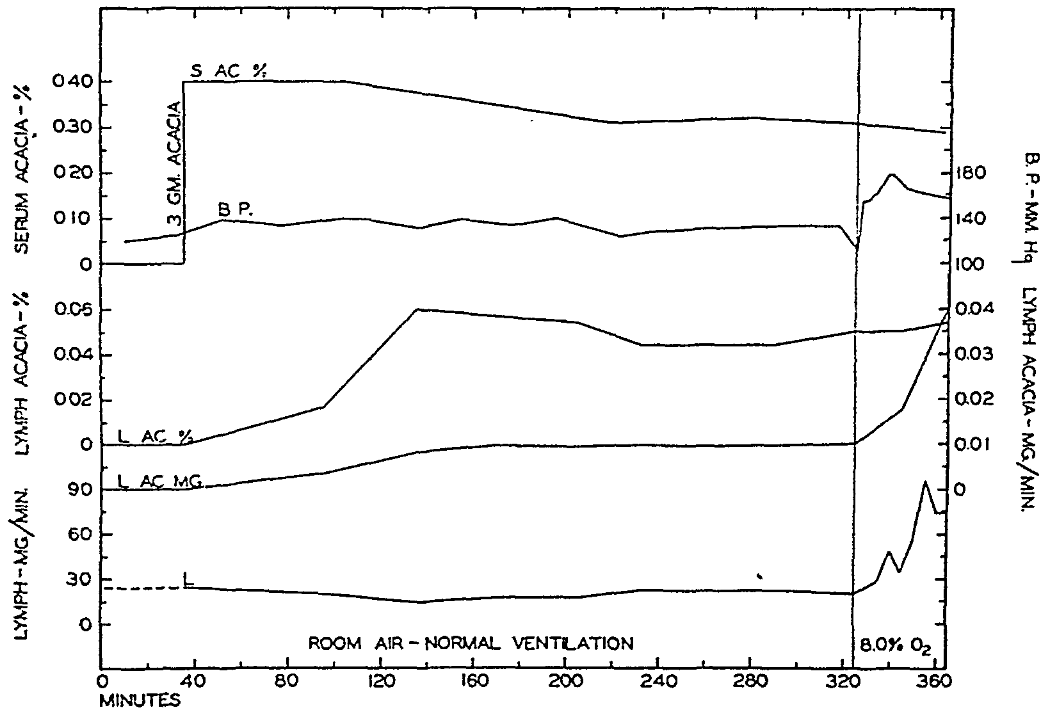


Fig. 1. Normal cervical lymph flow, followed by exposure to 8.0 per cent oxygen. Passage of intravenously injected acacia from blood to lymph. Increased capillary permeability with low oxygen shown by increased output of lymph acacia.

exposed to oxygen-nitrogen mixtures containing from 8.0 to 10.5 per cent of oxygen, though in a few cases mixtures containing as little as 4.0 per cent were used.

The experiment illustrated in figure 1, though performed for a different purpose, demonstrates very clearly the strikingly uniform flow of cervical lymph which can be obtained by this so-called "nodding dog" technique. Over a period of 5 hours and 25 minutes this dog was made to inspire adequate volumes of room air from the respiration pump. Throughout this period the average lymph flow was 19.9 mgm. per minute, the average deviation being only 2.4 mgm. per minute. At the end of this long control period, the animal was exposed to a gas mixture containing 8.0 per cent

oxygen. Immediately the flow of lymph began to increase, and continued to do so for 30 minutes when the peak flow was reached. This peak amounted to 4.8 times the normal flow. Though the administration of the low oxygen was continued, the lymph flow decreased during the next 10 minutes, following which the experiment was terminated.

Figure 2 illustrates a typical experiment in which the blood gases, arterial pressure, and blood and lymph proteins were determined as well as the

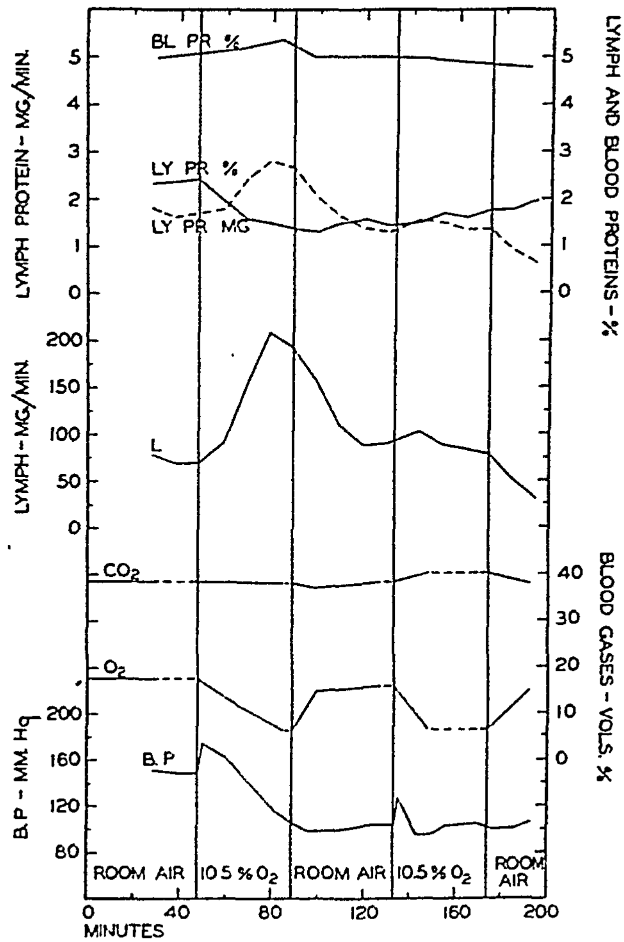


Fig. 2. Cervical lymph flow, lymph and serum proteins, blood gases, and arterial blood pressure of a dog alternately exposed to room air and 10.5 per cent oxygen.

lymph flow. During a control period lasting for about 50 minutes, the animal received room air from the respiration pump and, as the illustration shows, everything remained quite constant. At the end of this period the animal was shifted to a mixture containing 10.5 per cent oxygen, exposure to this mixture continuing for the next 41 minutes. During the first 2 minutes of low oxygen the arterial blood pressure rose 27 mm. of mercury, and then fell gradually and steadily until the end of the period, when it had reached a value of 100 mm. of mercury. The control blood pressure

was 150 mm. of mercury. The blood oxygen began to decrease immediately from its control value of 17.7 volumes per cent, and continued to decrease until at the end of the period it had reached 6.5 volumes per cent. The blood carbon dioxide remained practically constant throughout the entire experiment. Immediately upon the shift to 10.5 per cent oxygen, the lymph flow began to increase from its control level of 71 mgm. per minute. This increase continued until at the end of 31 minutes it had reached 208 mgm. per minute, or approximately 3 times the control value. Though the low oxygen continued for another 10 minutes, this peak flow of lymph was not maintained, but fell during this time approximately 15 mgm. per minute.

The animal was then shifted back to room air. The blood oxygen returned to approximately the normal value, and the lymph flow returned gradually to within 16 mgm. per minute of the control level. The flow remained constant at this figure for 13 minutes, when the animal was shifted again to the 10.5 per cent oxygen mixture. The arterial blood pressure again showed the short sharp rise which is characteristic of exposure to low oxygen. This rise subsided quickly and the blood pressure remained fairly constant for the duration of the experiment. The blood oxygen fell, as during the previous exposure, to 6.5 volumes per cent. During the first 11 minutes of this second exposure to low oxygen, the lymph flow increased only 17 mgm. per minute, or 1.2 times the normal value. The low oxygen was continued for 30 more minutes, during which time the lymph flow gradually subsided to approximately the control level. At this point the animal was shifted back to room air, and for 20 minutes the flow continued to fall until it was 40 mgm. per minute below the control level.

A total of 9 animals were similarly exposed to low oxygen. In each of these animals lymph flow was increased. Not all, however, responded to this treatment exactly as did the animal of figure 2. The range of increases was from 1.2 to 4.8 times the control flow. There appeared to be differences in the susceptibility of different animals to this treatment. For example, one dog reached a peak flow of 2.6 times the normal in 10 minutes; while another reached a peak flow of only 1.4 times in 97 minutes. A few of these 9 animals were exposed to low oxygen a number of times with an adequate interval of room air between exposures. The results of such repeated exposures can be summarized as follows:

1. After a short initial exposure to low oxygen a second exposure to the same concentration of oxygen will, in the majority of cases, bring about a second increase in flow which will invariably be less than the initial increase. Figure 2 shows the usual effect of such a second exposure. In some cases, however, the second exposure either will cause no increase at all or may be responsible for a decreased flow.

2. Following a short initial exposure, a second exposure to a gas mixture containing less oxygen than that of the first will in some animals produce a flow nearly equal to and occasionally greater than the initial increase. Here again, however, some animals will not respond or will show a decrease.

3. Following a long exposure (over 40 min.) to low oxygen, it becomes increasingly difficult to produce a secondary increase in lymph flow. In some cases following long exposure, mixtures containing as little as 4.0 per cent oxygen had little or no effect on the flow other than to cause some retardation.

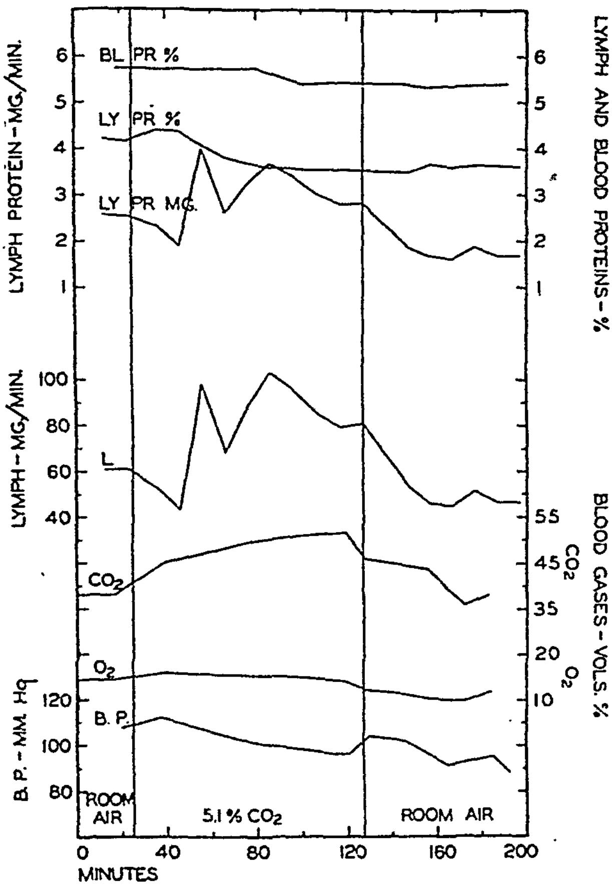
It has already been mentioned that once the maximum flow has been reached, continued exposure to the low oxygen mixture does not cause this high rate of flow to persist. Even though the animal continues to breathe the low oxygen mixture for many minutes beyond the peak, the flow will fall considerably below the maximum, in many cases returning to or nearly to the control level within the period of exposure. In one experiment it was found that the flow of thoracic duct lymph increased when the animal inspired low oxygen mixtures, just as the cervical flow increased. Gesell (1928) reported a similar finding. Certainly, then, exposure to low oxygen tensions must cause similar increases in all of the lymphatic channels of the body. A two- or three-fold increase in the lymph flow in the entire lymphatic system must represent a tremendous loss of fluid from the circulating blood in a very short period of time. Since this is undoubtedly true, the consequent increase in the colloid osmotic pressure of the blood serum (Landis, 1927–1928) would be sufficient to prevent further loss of fluid, even in the face of the damage to the vascular membranes brought about by the low oxygen saturation of the blood. It is easy to understand, therefore, that further exposure of the animal to low oxygen will have little or no effect upon lymph production, and in many cases will result in a diminished flow.

In a few experiments lymph flow remained somewhat higher than the control level, even after the animal had been shifted back to room air for a considerable length of time. Such a finding might be taken to indicate that some permanent damage had been done to the capillaries, and even though the colloid osmotic pressure was higher than normal the damage may have been great enough to permit a continuous leaking of fluid and protein from them.

Underventilation and exposure to high carbon dioxide mixtures. Early in this series of experiments 3 dogs were exposed to carbon dioxide-oxygen-nitrogen mixtures containing 8.9, *5.1* and 17.0 per cent of carbon dioxide, respectively. The arterial blood pressure showed a sharp initial rise, after which it returned approximately to the normal value where it remained fairly constant. The blood oxygen showed an average increase

of 1.4 volumes per cent, while the carbon dioxide increased 2.6, *13.7* and 15.2 volumes per cent, respectively. In each case there was an almost immediate increase in lymph flow, amounting in the respective animals to 2.8, *1.7* and 2.1 times the normal rate.

Figure 3 is an illustration of the findings from the second of these experiments, the exact values for which appear in italics in the preceding



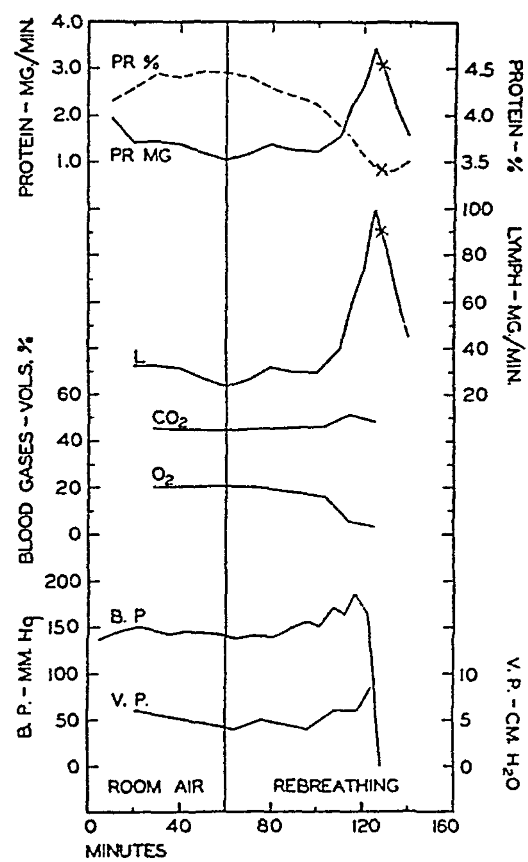


Fig. 3 Fig. 4

Fig. 3. Cervical lymph flow, lymph and serum proteins, blood gases, and arterial blood pressure of a dog exposed to 5.1 per cent carbon dioxide.

Fig. 4. Cervical lymph flow, lymph protein, blood gases, and arterial and venous blood pressures of a dog rebreathing from an 80-litre spirometer until circulatory collapse.

paragraph. With regard to lymph flow the results of these three experiments are not unlike those of the low oxygen experiments.

At the beginning of each of the 20 experiments of this series, a control period was run during which the animal breathed room air delivered by the respiration pump. In adjusting the pump so that the animal received adequate ventilation, it was discovered that the blood carbon dioxide could

be increased without significantly lowering the oxygen content by the simple expedient of decreasing the volume of air delivered. Five animals were subjected to this treatment. The output of the pump was first adjusted so that the air delivered to the animal was adequate to maintain the normal oxygen and carbon dioxide content of the blood. This was determined by analyzing arterial blood samples. After a short preliminary period the inspiratory volume was decreased anywhere from 17 to 58 per cent. The blood oxygen showed only insignificant changes, the greatest decrease observed being only 3.7 volumes per cent. In every instance the carbon dioxide content of the blood increased, the smallest increase observed being 2.0 volumes per cent, the largest 8.9 volumes per cent. As a result of this treatment each animal responded with an increased production of cervical lymph. These increases ranged from 1.3 to 3.0 times the normal flow.

Here again there appeared to be differences in the susceptibility of the animals to the increased carbon dioxide. In one instance it required only 25 minutes to increase the flow 3 times, while in another it required 40 minutes to attain an increase of 2.4 times, and in still another 30 minutes were required to bring about an increase of only 1.3 times.

Following this treatment, the animals were given normal ventilation for sufficient time to reduce their blood carbon dioxide to the control level, after which they were exposed to low oxygen mixtures. In each case there was a second increase in the lymph flow which was equal to or greater than the increase resulting from the underventilation. This observation would seem to indicate that the damage caused by increasing the carbon dioxide load of the blood was less extensive and of much shorter duration than that caused by decreasing the oxygen saturation, for, as already pointed out, it is practically impossible to increase the flow of lymph after an initial treatment with low oxygen.

Having observed the effect of increased carbon dioxide, there appeared the possibility that the increased lymph production resulting from a deficient oxygen supply was not due alone to anoxemia but was the combined result of decreased oxygen and increased carbon dioxide. Careful checks of the blood gas analyses of the low oxygen experiments showed that in only one case had the carbon dioxide content of the blood risen more than 1.0 volume per cent during the period of the increased flow. All of the other experiments showed that the carbon dioxide had remained at the control level or had fallen slightly below. It is safe, therefore, to say that anoxemia alone is capable of producing the changes observed following exposure to low oxygen.

Rebreathing experiments. In order to determine exactly the degree of oxygen desaturation necessary to increase lymph production, 6 rebreathing experiments were performed. By making use of this procedure it was

possible to reduce the blood oxygen gradually over a long period of time. By repeatedly analyzing the blood gases, the desaturation curve was drawn and compared with the lymph production curve. Figure 4 illustrates the results of one of these experiments. Careful analysis of the data revealed that the flow of lymph began to increase when the oxygen saturation reached 70.7 per cent of normal. As the rebreathing continued the blood oxygen saturation decreased more rapidly, being accompanied by an increasingly rapid lymph production. When the slope of the lymph curve became steepest, the saturation of the blood was only 46.3 per cent. The maximum flow was 3.3 times normal, and at this time the oxygen saturation was only 14.6 per cent. Four minutes after attaining the maximum flow, the oxygen supply was so completely diminished that the animal died. It is interesting to compare the oxygen saturation of this blood when lymph flow began to rise, with the chart of oxygen saturation in relation

TABLE 1

Relation of cervical lymph flow to blood oxygen saturation and altitude

EXPERIMENT	INCREASED FLOW		STEEPEST FLOW		MAXIMUM FLOW	
	O_2 saturation	Altitude	O_2 saturation	Altitude	O_2 saturation	Increase over normal
	per cent	*feet*	*per cent*	*feet*	*per cent*	*times*
1	70.7	18,500	46.3	>20,000	14.6	3.3
2	76.7	16,500	62.5	>20,000	17.0	3.2
3	82.6	14,000	75.9	17,000	69.0	3.9
4	69.8	19,000	40.7	>20,000	12.5	1.8
5	74.5	17,500	45.2	>20,000	36.0	1.6
6	75.5	17,000	44.3	>20,000	13.0	2.6
Average...	75.0	17,000	52.5	>20,000	27.0	2.7

to altitude published by Dill, Bock, Edwards and Kennedy (1936). According to their figures, this particular animal had reached an altitude equivalent to 18,500 feet.

Table 1 shows the results of the 6 rebreathing experiments. Inasmuch as 14,000 feet is generally considered to be the altitude at which most mountain climbers and other high altitude workers are first seriously affected by oxygen lack, it is interesting to note that the average saturation of these 6 animals was 75.0 per cent, which is roughly equivalent to an altitude of 17,000 feet. Since the susceptibility of these dogs varied widely, the range being approximately from 14,000 to 19,000 feet, it should be quite safe to say that the critical saturation with regard to lymph flow was roughly equal to 14,000 feet also. To continue this analogy, it is interesting to note that the average saturation when the slope of the flow curves was greatest is 52.5 per cent, which is equivalent to an altitude greater

than 20,000 feet, beyond which it is very difficult for man to venture without the aid of oxygen.

The effect of low oxygen on cardiac lymph flow. One experiment was performed in which both the cervical and cardiac lymph flows were observed during exposure to low oxygen mixtures. The cannulation of the cardiac lymphatic duct has been described by Drinker, Warren, Maurer and McCarrell (1940), and except for this addition, the procedure was no different from that used in the other low oxygen experiments. Figure 5

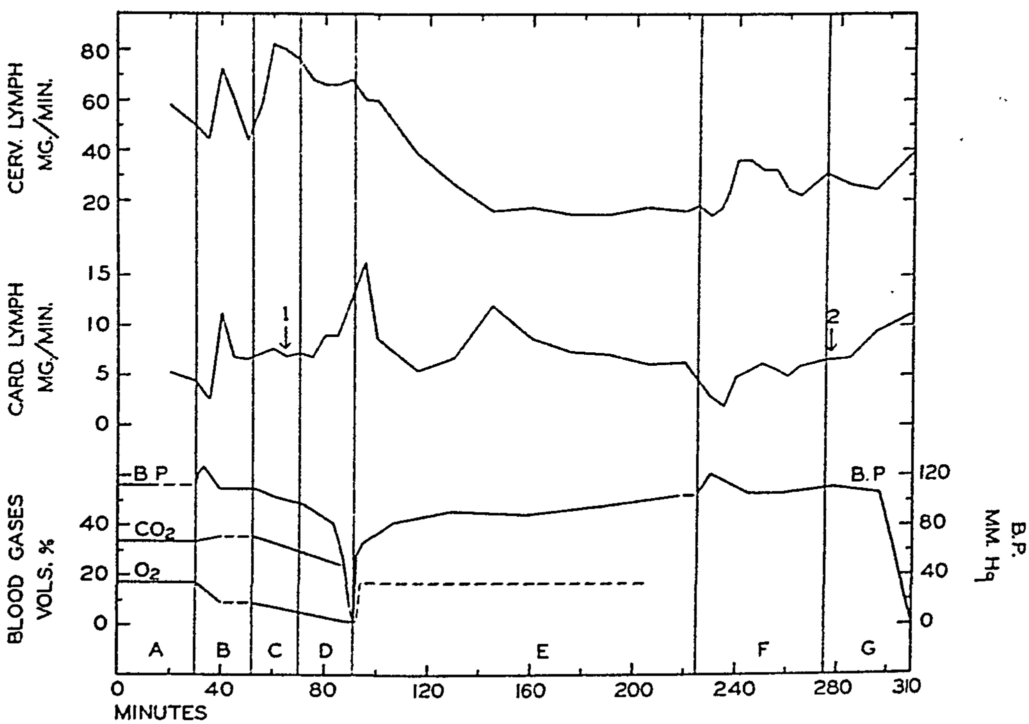


Fig. 5. Cardiac and cervical lymph flows, blood gases, and arterial blood pressure of a dog during exposures to low oxygen and to 100 per cent oxygen. *A*, room air control; *B*, 8.0 per cent oxygen; *C*, 6.0 per cent oxygen; *D*, 4.5 per cent oxygen; *E*, 100 per cent oxygen; *F*, 6.0 per cent oxygen; *G*, 4.5 per cent oxygen. At arrow *1*, cardiac lymph became slightly bloody and remained so until, at arrow *2*, it became very bloody.

illustrates the results. In general the flow of cardiac lymph followed much the same course as that of the cervical lymph, showing an initial increase of 2.5 times on exposure to 8.0 per cent oxygen and a four-fold increase with 4.5 per cent oxygen. There was observed, however, one striking difference. During period *D* of figure 5, the animal was exposed to 4.5 per cent oxygen for 21 minutes, causing the arterial blood pressure to fall to only 10 mm. of mercury. At this point the animal was shifted to 100 per cent oxygen, which was continued for 2 hours and 14 minutes. Section *E* illustrates the flows during this time, and it is during this period that

the difference is noted. The flow of cardiac lymph maintained a minimum rate which was never less than 1.4 times the control level, while the cervical flow fell considerably below the control level and remained there. It seems possible that the decreased oxygen supply had a more damaging influence on the capillaries of the heart than those of the region drained by the cervical ducts. This is substantiated not only by the increased flow maintained throughout section *E*, but also by the fact that at arrow *1*, the cardiac lymph became slightly bloody and remained so throughout the experiment. Further exposure to low oxygen (sections *F* and *G*) caused the lymph flows to increase again. During this time (arrow *2*) the cardiac lymph became more bloody than before, while the cervical lymph still showed no change in color. The appearance of red cells and hemoglobin in the cardiac lymph must certainly denote a high degree of cardiac capillary damage.

Lymph protein. Total protein was determined by means of the Zeiss dipping refractometer calibrated against known samples of dog serum and lymph, and frequently checked by micro-Kjeldahl determinations. The data are in accord with findings reported many times from this laboratory. As long as lymph flow remains constant, the percentage protein in the lymph also remains constant; and when lymph flow increases, the percentage of protein decreases, returning to the original value when the flow returns to normal. However, when lymph protein is calculated in milligrams per minute (lymph flow in milligrams per minute × per cent protein), it is found that the output of protein varies directly with the rate of lymph flow. Figures 2, 3, 4, 6 and 7 clearly illustrate this relationship.

In contrast with the statement of Landis (1927–1928) that carbon dioxide allowed the escape of none of the plasma proteins into the capillary filtrate, the present work shows that both increased carbon dioxide and decreased oxygen have similar and equal effects upon the protein content of cervical lymph.

This augmented output of protein in the lymph during exposure to low oxygen or high carbon dioxide is believed to indicate that the permeability of the capillary walls is greatly increased. Further indication of increased permeability is shown by the fact that while the lymph flow and lymph protein output are increasing, there is a simultaneous decrease in the concentration of the serum proteins. This is clearly illustrated in figures 2 and 3.

Lymph albumin and globulin. Drinker, Warren, Maurer and McCarrell (1940) showed that the albumin/globulin ratio of cardiac lymph was, in most of their experiments, approximately equal to the ratio in the blood of the same animal. In the present work the same was found to be true of the albumin/globulin ratios of cervical lymph. To determine the effect of exposure to low oxygen or high carbon dioxide and the resulting increase

in lymph flow upon the albumin/globulin ratio of cervical lymph, these protein fractions were determined at frequent intervals throughout 6 experiments. Albumin was determined by precipitating the globulin with 22.5 per cent sodium sulphate, filtering off the precipitate, and making nitrogen determinations on the filtrate by micro-Kjeldahl analysis, a modified Pregl apparatus being used for the distillation. Globulin was determined by difference.

In each of these experiments it was found that the albumin/globulin ratio, calculated from the output of each in milligrams per minute, was

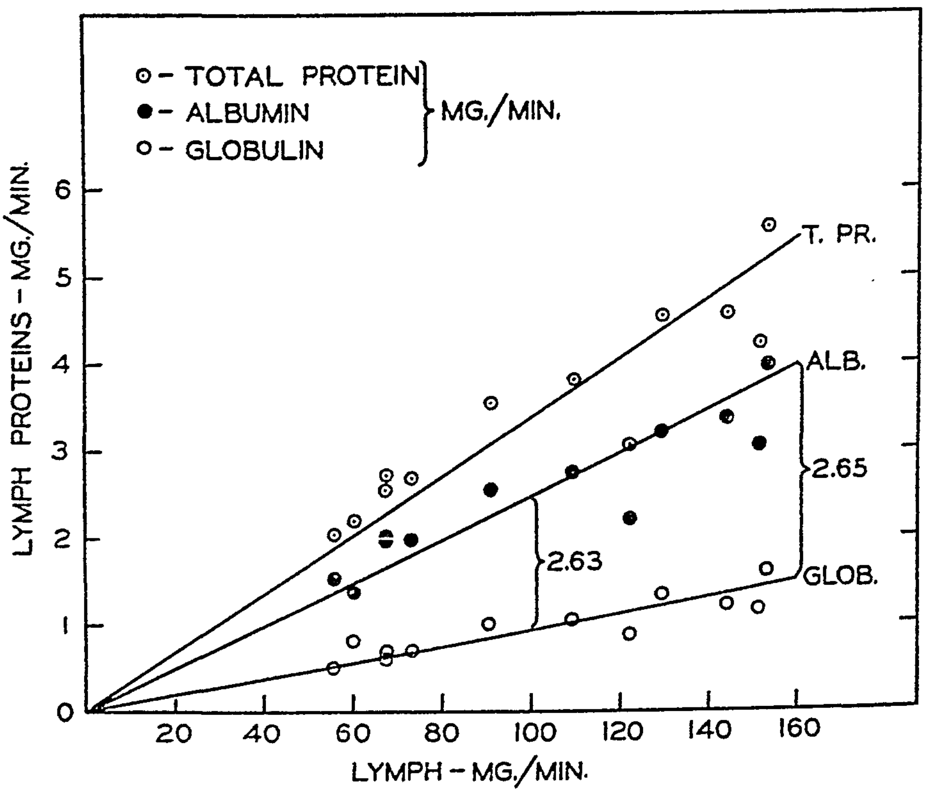


Fig. 6. The relation of cervical lymph protein, albumin, and globulin in milligrams per minute to the rate of lymph flow in milligrams per minute. Flow was increased in this dog, first by underventilation and later by rebreathing.

constant regardless of the rate of lymph flow. Figure 6 shows this constant relationship in one experiment in which lymph flow was made to vary between 56 and 154 mgm. per minute.

Acacia experiments. In 6 of the present experiments 3 grams of gum acacia (50 cc. of a 6 per cent solution in saline) were injected intravenously, and for the duration of the experiments samples of blood and lymph were analyzed for acacia as described by Maurer, Warren and Drinker (1940). Maurer *et al.* have stated that it is their belief that the protein of extra-

cellular fluids is derived from the protein of the circulating blood. To substantiate this belief, they quoted experiments in which the passage of acacia and horse serum was followed from the blood stream into the pericardial and peritoneal fluids of dogs. Drinker, Warren, Maurer and McCarrell (1940) utilized the passage of these foreign substances from the blood stream into cardiac lymph to demonstrate the permeability of the cardiac blood capillaries. The present acacia experiments were performed not only to shed further light upon the source of lymph protein, but to substantiate the belief that the permeability of blood capillaries is increased as a result of decreased blood oxygen or increased blood carbon dioxide.

In the experiment illustrated in figure 1, the passage of acacia from the blood stream into the cervical lymph was demonstrated. The amount of acacia appearing in the lymph reached a maximum value of 0.01 mgm. per minute after 2 hours and 17 minutes, and remained at this level for another 3 hours and 8 minutes. It is significant that until this steady level of lymph acacia had been reached, the acacia in the blood stream decreased steadily and then it, too, remained constant. At the end of this long control period, anoxemia was induced by exposing the animal to 8.0 per cent oxygen. Coincident with the increased lymph flow, there was an immediate and sharp rise in the output of acacia into the lymph to 0.04 mgm. per minute, the percentage of lymph acacia remaining practically constant. In contrast to this increased output of acacia into the lymph there was a further decrease in the concentration of serum acacia.

Figure 7 illustrates another of these acacia experiments in which lymph protein and lymph acacia were followed simultaneously. Three distinct increases in lymph flow were produced, the first and third (sections *B* and *F*) by exposure to 8.0 and 7.0 per cent oxygen, respectively, and the second (section *D*) by a 58 per cent reduction of the inspiratory volume. The acacia was administered to this animal 49 minutes before the data shown in figure 7 were obtained. During that time the output of lymph acacia had almost reached a steady state. As in all the other experiments the percentage of lymph protein decreased coincident with the increased rate of flow, while the output of protein in milligrams per minute increased, both of these values approaching normal as the rate of flow decreased. It is significant that the acacia curves of this figure, both for percentage and for output in milligrams per minute, very nearly parallel the protein curves..

These experiments not only demonstrate that the blood capillaries are normally permeable to acacia and to protein, but show also that anoxemia and high blood carbon dioxide can greatly increase this permeability. Also, having observed that coincident with an increased rate of lymph flow there is an increase in the output of protein and acacia into the lymph (figs. 1, 2, 3, 4, 6 and 7) and a decreased concentration of these substances

in the blood stream (figs. 1, 2 and 3), there can be little doubt that the normal protein constituents of lymph are derived from the protein of the circulating blood.

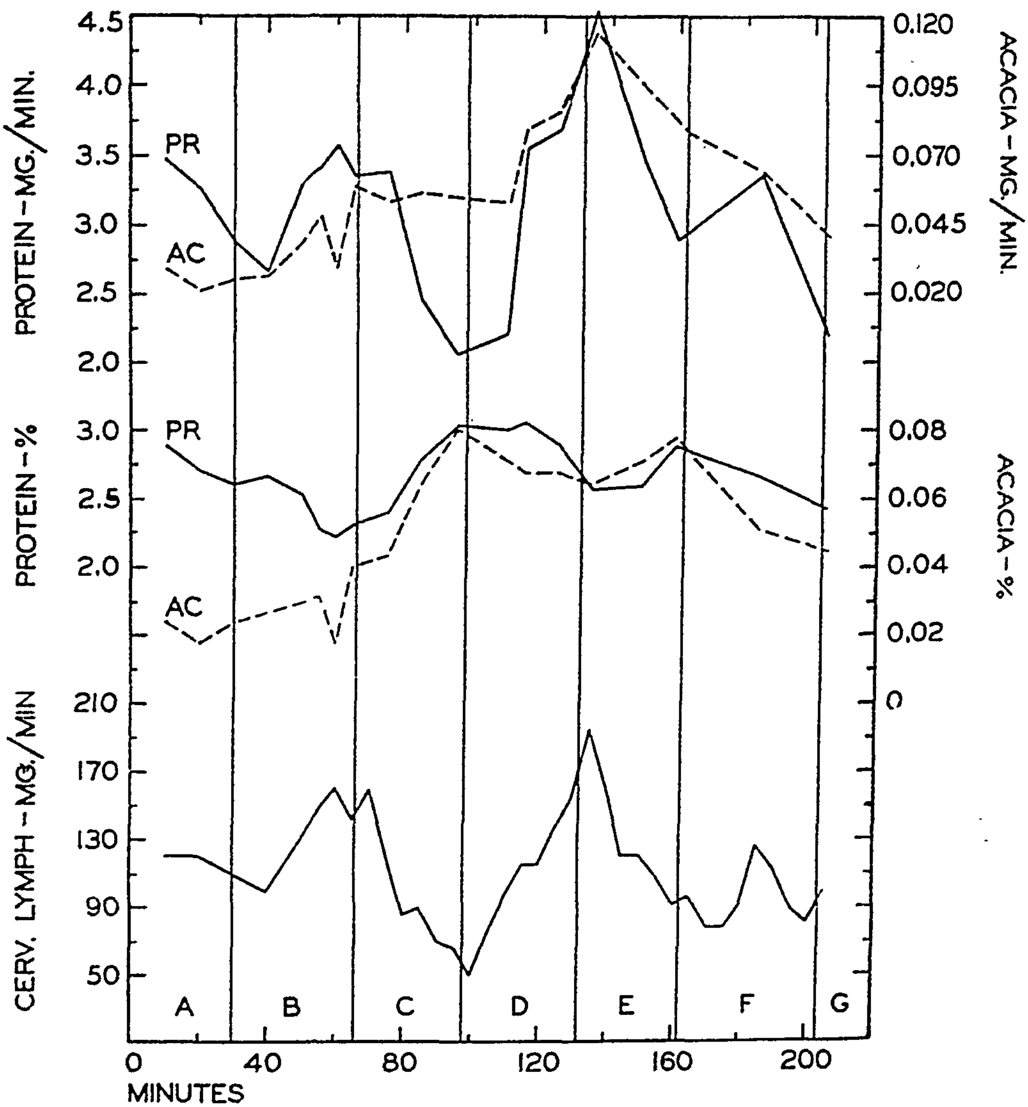


Fig. 7. Per cent lymph protein and lymph acacia, output of lymph protein and lymph acacia in milligrams per minute, and cervical lymph flow of a dog during exposure to low oxygen and during underventilation. *A*, room air control; *B*, 8.0 per cent oxygen; *C*, room air; *D*, inspiratory volume decreased 58 per cent; *E*, normal ventilation; *F*, 7.0 per cent oxygen; *G*, room air. Three grams of acacia (50 cc. of a 6.0 per cent solution) were injected intravenously 49 minutes prior to the beginning of section *A*.

Discussion. In each of the 20 experiments reported here, the arterial blood pressure was recorded at frequent intervals. Figures 1, 2, 3, 4 and 5 illustrate very clearly the effects of exposure to low oxygen and to high

carbon dioxide upon this pressure. The general effect is that of a short and rapid increase averaging 30 mm. of mercury and in these experiments never exceeding 51 mm. of mercury. In 6 of these experiments the venous blood pressure was measured in the external jugular vein. Here, the effect of the exposures ranged from a fall of 2.6 cm. of water to a rise of 5.4 cm. of water. Figure 4 shows the venous pressure recordings of one of these experiments. The question may arise as to whether these relatively small increases in arterial and venous blood pressures may have themselves been responsible for the increases in lymph flow that were observed. Haynes (1932) has shown that the arterial pressure of dogs was increased 3 times before there appeared any increase in the flow of subcutaneous lymph. McMaster and Hudack (1932) have reported that the permeability of the capillaries of the frog's web was increased only when the venous pressure approximated that in the small arteries. Krogh, Landis and Turner (1932) have stated that fluid accumulated in the tissue spaces of the human arm only when the venous pressure exceeded 15 to 20 cm. of water. Since the greatest increase in arterial pressure recorded was only 1.4 times the normal and since the highest venous pressure observed was 11.3 cm. of water, it seems very improbable that the pressure changes observed in these experiments were in any way responsible for the increased production of lymph accompanying exposure to low oxygen or high carbon dioxide. It is also significant that the venous pressure rise does not occur until some minutes after a considerable increase in lymph flow has been attained.

Several of the animals were exposed to low oxygen or made to rebreathe until the circulation had almost completely collapsed. At this point some were allowed to die while others were shifted to 100 per cent oxygen for varying lengths of time. Immediately the blood pressure rose to or nearly to the normal level. Though all of these animals had been exposed to low oxygen longer than 40 minutes (48 to 100 min.), it is interesting to note the varied responses in lymph flow after the shift to 100 per cent oxygen. Two of the animals were exposed to 100 per cent oxygen for 33 and 13 minutes, respectively, the flow of the first remaining much higher than normal, the second actually showing an increase of 59 mgm. of lymph per minute. The cardiac lymph flow of a third animal (shown in fig. 5) remained higher than normal and even showed transient increases during 2 hours and 14 minutes of 100 per cent oxygen. In contrast to these results, 2 other animals were given this treatment for 60 and 70 minutes, respectively, the flow of the first falling below normal, that of the second falling just to normal, while the cervical flow of the animal of figure 5 also fell considerably below normal. From so little and such widely varying data it is impossible to judge what the real effect of the 100 per cent oxygen was. Perhaps the 3 decreased flows observed were simply the natural result of

the increased osmotic pressure which followed the great loss of fluid from the circulating blood. There can be no doubt that 100 per cent oxygen did not have the beneficial effect upon the cardiac capillaries that it appeared to exert upon the capillaries of the region drained by the cervical ducts in the experiment of figure 5. The real significance of such treatment cannot be determined until similar experiments are performed during which the animal is exposed to pure oxygen for periods of many hours.

The question of acclimatization to the low oxygen tensions of high altitude has intrigued many physiologists. Talbott and Dill (1936), in describing their observations of persons living at the altitude of 17,500 feet in the mountains of northern Chile, report that these people, though constantly exposed to an atmosphere with a decreased oxygen pressure, appear to be normal in most respects except for the fact that the average arterial oxygen saturation of those observed was 75 per cent. It is interesting to note that the arterial saturation of these people reported by Talbott and Dill is the same as the average saturation at which lymph flow began to increase in the present experiments. Perhaps these dogs would have become acclimatized to low oxygen if exposed for sufficiently long periods of time, and if such were the case, it is possible that increased lymph flows would not have been observed. One of the present experiments is perhaps significant in this respect. This experiment was performed upon a dog weighing 11 kgm., from which had been drawn approximately 1500 cc. or more of blood over a period of about 4 weeks. At the time of the experiment the animal appeared quite normal, though upon analysis of her blood there were found only 8.7 volumes per cent of oxygen. This animal rebreathed over a period of 2 hours, until the blood oxygen had dropped to 4.8 volumes per cent. During the first 40 minutes of the rebreathing, lymph flow decreased 8 mgm. per minute; during the next 50 minutes, the flow returned to the original value; and from that time on, during the next 30 minutes, the flow decreased steadily to a level of 16 mgm. per minute below the control. It is remarkable that this dog, whose arterial saturation was less than 50 per cent, appeared to be completely normal. It is not impossible that this animal had become acclimatized to this greatly decreased arterial saturation, and therefore did not respond on exposure to air low in oxygen as did the 19 other dogs of this series of experiments.

Interesting also in relation to the present work is the report of Graybiel, Missiuro, Dill and Edwards (1937) on experimental asphyxiation in cardiac patients. They conclude "that many cardiac patients are endangered when the oxygen of the inspired air falls to 12 per cent, which would correspond to an elevation of 14,500 feet. The untoward effects observed may be due to the general unfitness which is so often associated with heart disease or due more directly to embarrassment of the heart itself." The

experiment of figure 5 is perhaps significant in this respect, for here was seen in the heart of a normal dog a four-fold increase in the production of cardiac lymph as well as the appearance of erythrocytes in this fluid after a relatively short exposure to low oxygen. If exposure to low oxygen can produce such long-lasting changes in the lymph production of a normal heart, it is not hard to understand that the heart of a cardiac patient might be embarrassed under similar circumstances.

Perhaps, too, some of the symptoms that appear on rapid ascents to altitudes of 14,000 feet or over, or during the exposure of unacclimatized individuals to low oxygen tensions, may in part be due to the great loss of fluid from the circulating blood, observed in the form of increased lymph production, and to the accompanying increase in the colloid osmotic pressure of the blood serum.

The author takes this opportunity to thank Miss Anne C. Messer for technical assistance in gas analysis.

SUMMARY

Experiments are reported in which dogs were exposed to low oxygen tensions and to increased tensions of carbon dioxide. Such treatment results in either case in the increased production of cervical lymph.

Following initial exposure to low oxygen, it becomes increasingly difficult to produce secondary increases in lymph flow by further exposures. However, initial exposure to increased carbon dioxide has little or no effect upon the production of secondary increases in lymph flow by exposure to low oxygen.

Rebreathing experiments showed that increased lymph production began when the arterial oxygen saturation reached 75 per cent, which is equivalent to an altitude of 17,000 feet, and that the production of lymph became greatest when the arterial saturation reached 52.5 per cent, which is equivalent to an altitude greater than 20,000 feet.

It was shown that the damage to cardiac blood capillaries was significant as reflected by greatly increased flow of cardiac lymph, the persistence of this increased flow even after long exposure to pure oxygen, and the appearance of erythrocytes in the lymph.

Without exception the percentage of protein in the lymph decreased with increased flow and returned to normal when the flow subsided. The passage of protein from the blood capillaries to the lymph, calculated in milligrams per minute, increased as flow increased and decreased as flow subsided. Coincident with increased output of lymph protein there was a decrease in the concentration of serum protein.

Acacia, injected intravenously, regularly appeared in the lymph. Equilibrium between serum acacia and lymph acacia was attained in from 40 minutes to 2 hours. With increased lymph flow, the course of lymph and serum acacia paralleled that of the lymph and serum proteins.

It was shown that the ratio between lymph albumin and lymph globulin remains constant with all rates of lymph flow.

The increased passage of protein and acacia from the blood stream into the lymph is believed to indicate that low blood oxygen and increased blood carbon dioxide result in increased capillary permeability with loss of fluid and protein from the circulating blood. This loss of fluid leads to increased colloid osmotic pressure of the blood serum.

The possible implications of this increased lymph flow are discussed.

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A QUANTITATIVE STUDY OF ACID IN THE INTESTINE AS A STIMULUS FOR THE PANCREAS

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The fact that acid in the intestine causes the pancreas to secrete has been known for almost half a century (Bekker, 1893). Literature dealing with the subject has been reviewed by Pavlov (1910), Babkin (1914), and Ivy (1930). Although acid was one of the first pancreatic secretagogues discovered, its physiological significance is still uncertain. We do not know how much acid must be present in the intestine to stimulate the pancreas nor how acid the intestinal contents may become during digestion.

An attempt has been made by one of us (Thomas, 1940) to determine the maximal acidity of the intestinal contents of the dog during the digestion of raw meat. Our purpose in the present investigation was to ascertain the minimal acidity that would suffice to stimulate the pancreas. By determining the acid threshold (in pH units) for comparison with the acidity observed in the intestine, we hope to provide a basis for estimating the significance of acid as a stimulus for pancreatic secretion during normal digestion.

METHOD. The method that we used has not previously been applied to the study of pancreatic function although a similar but less convenient procedure was described by Tuckerman (1883). Dogs were provided with gastric and duodenal fistulae fitted with large cannulae (minimal internal diameter $\frac{5}{8}$ in.) as described previously (Thomas and Crider, 1934). For these studies the duodenal fistula was placed at the level of the main pancreatic duct in the duodenal wall directly opposite the opening of the duct.

Instead of attempting to cannulate the duct through the fistula-tube as Tuckerman did, we collected the secretion during the periods of observation through a funnel-shaped rubber cup which was held lightly against the duodenal mucosa surrounding the duct by means of a conical coiled spring attached to the cannula. The arrangement is illustrated in figure 1. The wall of the cup was deeply grooved on the outside to provide drainage for extraneous fluids. Control experiments on an animal with the duodenal fistula below the level of the duct proved that contamination of the secretion with succus entericus was quantitatively unimportant.

Three dogs were used, in one of which the common bile duct was transplanted into the stomach and the accessory pancreatic duct ligated. Observations were made when the stomach and small intestine were empty, 18 to 24 hours after a small meal of selected lean meat.

The animal was supported in a standing position on a table by means of a comfortable muslin harness suspended from a horizontal rod. The secretion dripped into a funnel which was connected through a displacement bulb to a drop recorder of the Wesson (1933) type. This apparatus was filled with distilled water and leveled so that addition of fluid in the funnel caused an equal volume of water to drop from the pipette.

To prevent fasting gastric secretion (or bile in the animal with the transplanted duct) from entering the duodenum in the course of an experiment, the antrum and body of the stomach were drained through two tubes via the gastric fistula. The antral tube was attached to a suction apparatus which maintained a negative pressure of 20 to 30 cm. of water.

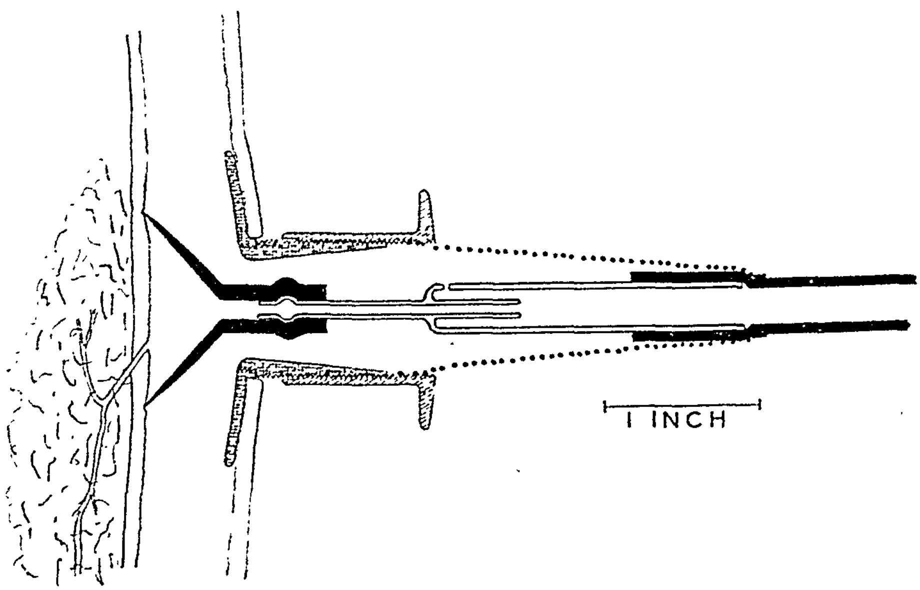

Fig. 1. Sectional view of the apparatus used for collecting pancreatic secretion through a duodenal fistula.

Two arrangements were used for putting test solutions into the intestine which we shall refer to as the injection method and the perfusion method, respectively. In the first a single injection tube was passed through the duodenal cannula to a point about 12 inches below the fistula. Solutions were injected in 20, 40 or 60 cc. amounts. For perfusion, an additional tube, larger and longer than the other, was passed to a point about 22 inches beyond the end of the injection tube. Solutions were delivered into the intestine through the shorter tube by a motor-driven pump at the rate of 15 cc. per minute and, after passing through the upper intestine,

drained out through the longer tube. The solutions were not confined to the regions below the point of injection. In all the experiments in which perfusion was used and in many of the others appreciable amounts of the solutions drained out through the fistula; hence the greater part of the duodenum was exposed to their action.

The solutions used were buffer mixtures of various acids and their sodium salts (the hydrochloride in the case of glycine) in different relative concentrations, depending on the pH. All solutions were 0.15 M except the citrate buffer which was 0.13 M. The stronger acids and the dibasic acids and their salts are approximately isotonic in 0.15 M solutions. The weaker acids are hypotonic in this concentration and to these NaCl was added to bring the Δ to between 0.5° and 0.6°C.

RESULTS. *pH thresholds.* The pH at which the various buffer solutions began to be effective in causing pancreatic secretion was not significantly affected by the method of administering the solution although a slightly higher percentage of positive results was obtained by perfusion than by the injection method. In this respect also the results were the same in the three animals, including the one in which bile was excluded from the duodenum. In table 1 the results are classified as "positive," "negative" or "doubtful." Those classified as "positive" were characterized by an unmistakable increase in the rate of pancreatic secretion which occurred within a reasonable time after the start of the injection or perfusion and for which no other cause than the applied stimulus was apparent. In those classified as "doubtful," an increase in secretion occurred but it was so slight as to be within the range of variation evident during the control period, or it occurred too early or too late to be interpreted as the result of the stimulus, or some other probable cause for the increase was evident. All others are classified as "negative."

The threshold may be defined for our purpose as the pH at which the ratio of positive to negative results becomes greater than 1. These ratios are given in the last column of the table. According to this definition the various thresholds were as follows: HCl, less than pH 3.0; phosphate, between pH 3.0 and pH 4.0; sulphanilic acid, between pH 4.0 and pH 4.5; glycine, between pH 4.0 and pH 4.5; lactate, between pH 4.5 and pH 5.0; acetate, above pH 4.5; glutamic acid, between pH 4.5 and pH 5.0; citrate, above pH 7.0 (?).

From the size of the ratios as given in the table as well as from the magnitude of the responses as shown in the records, it is evident that the threshold for lactate buffers is nearer pH 4.5 than pH 5.0; that for the glutamic acid buffers nearer pH 5.0. Although the citrate buffers were apparently effective in neutral solution, the effect was small and probably negligible. The threshold for citrate acting as an acid is probably not far from pH 5.0.

Differences were also evident in the amount of extra secretion produced by the different buffer solutions at the same pH. For example, consider-

TABLE 1

Summary of results

Explanation in the text

BUFFER	POSITIVE		NEGATIVE		DOUBTFUL		RATIO + TO −
	P*	I†	P	I	P	I	
NaCl + HCl							
N/500 HCl	3	10		5			2.6
N/1000 HCl		1	3	6			0.111
$NaH_2PO_4 + H_3PO_4$							
pH 3.0		7					∞
pH 4.0	1		7				0.142
Sulphanilic acid + NaOH							
pH 3.9–4.1		5				1	∞
pH 4.45–4.65	1	4	2	4	2	1	0.833
pH 5.0		1		3			0.333
Glycine + HCl							
pH 3.9–4.1	8				1		∞
pH 4.45–4.65	3		2	3	1		0.6
Lactic acid + NaOH							
pH 2.3–4.0		7		1			7.0
pH 4.45–4.65	9	35	2	13	1	3	2.93
pH 4.9–5.1		1	5	8			0.076
pH 6.9–7.2		1		5		1	0.2
Acetic acid + NaOH							
pH 4.45–4.65	3	3	1				6.0
pH 6.9–7.0				1			0
Glutamic acid + NaOH							
pH 3.2–4.0		10		1			10.0
pH 4.45–4.65	5	15		3		5	6.66
pH 4.9–5.1	3	4	3	6	1	8	0.77
pH 6.9–7.2				8			0
Citric acid + NaOH							
pH 4.45–4.65	12	7		2		3	9.5
pH 4.9–5.1	4		1	1	1		2.0
pH 6.9–7.2		3		1			3.0

* Perfusion.

† Injection.

ing only the positive results obtained by the injection method at pH 4.5, the lactate buffer produced on the average slightly less than 2 cc. of extra

secretion, the glutamic acid buffer slightly more than 3 cc. and the acetate buffer (3 experiments only) a little more than 3½ cc. Typical results obtained with lactic, glutamic and citric acid buffers at pH 4.5 are shown in figure 2.

In addition to the experiments listed in the table, HCl caused an increase in pancreatic secretion at pH 4.0 when buffered with colloidal

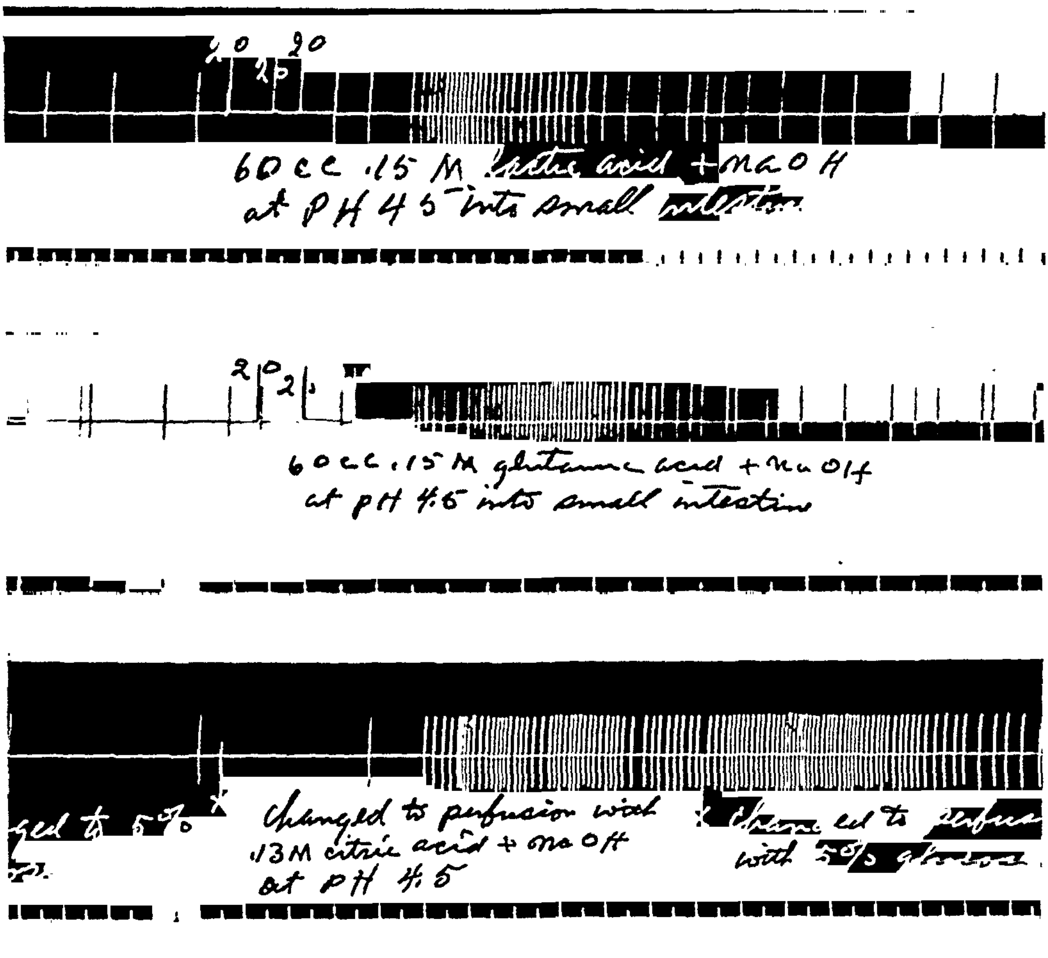

Fig. 2. Typical records showing the effect on pancreatic secretion of various buffer solutions in the intestine at pH 4.5. Upper record, injection of 60 cc. 0.15 M lactate; middle record, injection of 60 cc. 0.15 M glutamic acid buffer; lower record, perfusion for 10 min. with 0.13 M citrate. The drop recorder delivered 17 drops per cubic centimeter. Time is in 30 sec. intervals.

aluminum hydroxide and kaolin. Two proprietary preparations of aluminum hydroxide were used with similar results.

The buffering efficiency of the various solutions. Measurements of the pH of material that drained out of the intestine in perfusion experiments proved not only that the solutions were partially neutralized in the intestine but also that there was a marked difference in the amount of

neutralization of different buffer solutions. These considerations led to an investigation of the buffering capacity of the various solutions within the pH range found to be significant for our purpose. The results are shown in figure 3. A comparison of these titration curves with the data given in the table shows a close correlation between the buffering capacity of the solutions as indicated by the amount of NaOH required to cause a given

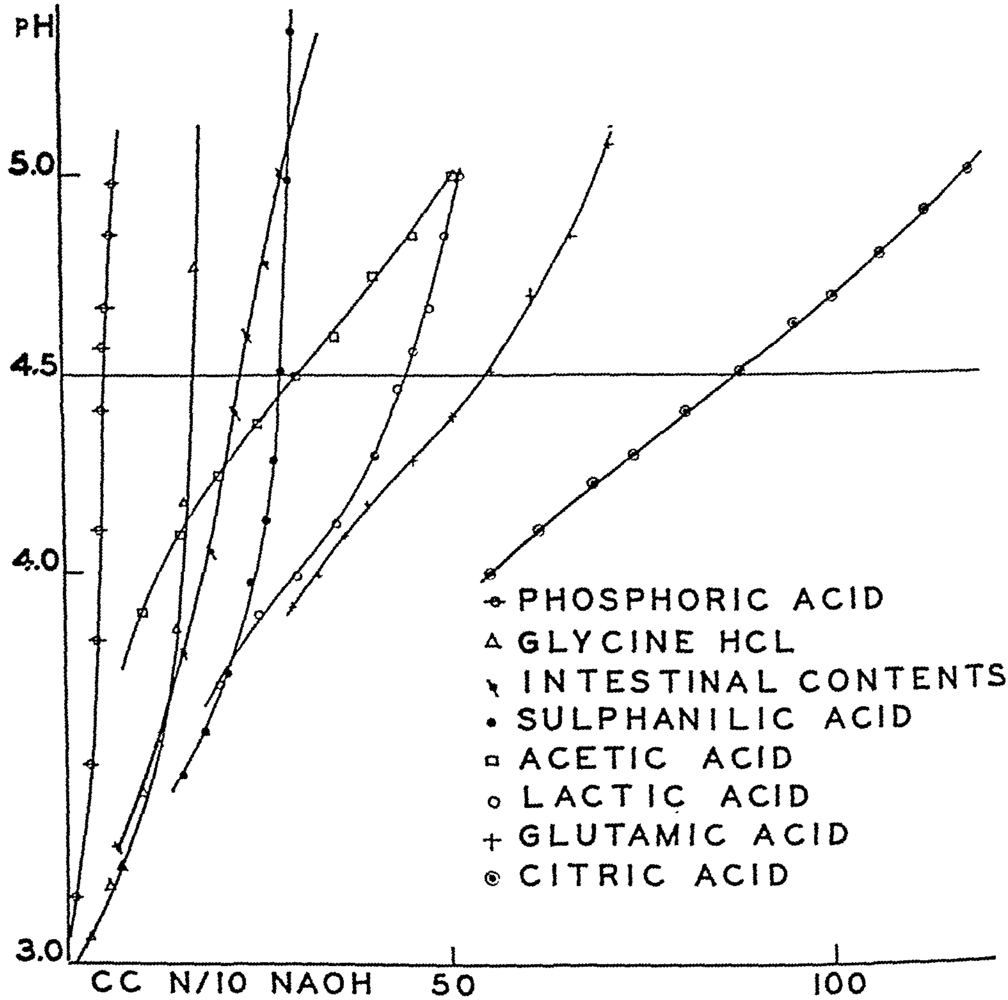

Fig. 3. Significant portions of titration curves of various acids. Fifty cubic centimeters of 0.15 M acid were adjusted to pH 3.0 and titrated to pH 5.0 or above with N/10 NaOH. The lower portions of some of the curves are omitted to avoid confusion. We are indebted to Earl Thomas, Jr. for most of these data.

change in pH and the pH at which they begin to act as stimuli for pancreatic secretion. It appears probable, therefore, that the apparent differences in threshold are caused by differences in the capacity of the solutions to maintain an effective degree of acidity in the intestine rather than to true differences in the threshold for acids of different molecular structure.

This interpretation is borne out by the behavior of glycine and acetic

acid, which are very similar in structure but differ greatly in the pH at which they begin to act as stimuli for the pancreas and differ in a corresponding manner in their efficiency as buffers. Sulphanilic acid, on the other hand, which belongs to an entirely different class of organic compounds is practically equivalent to glycine as a buffer and also as a stimulus for the pancreas within the pH range studied.

The periodic activity of the pancreas. We have made no special study of this phenomenon but find it necessary to refer to it because its occurrence affects the interpretation of our results. As pointed out by Boldyreff (1904) (for other references see Babkin, 1914) the periodic activity of the gastro-intestinal muscle, generally referred to at present as hunger activity, is accompanied by pancreatic secretion. In our dogs this apparently spontaneous secretion occurred in control experiments at intervals of 1½ to 2 hours. Its appearance tended to confuse the results in some instances, particularly when dealing with inert substances. Probably most, if not all, of the "positive" results recorded following the use of substances that were generally ineffective as stimuli are due to "periodic" activity. It is less of a factor in the results obtained with effective stimuli because most of the substances that cause pancreatic secretion also inhibit hunger activity, hence the secretion obtained is not likely to be augmented by "periodic" activity. Since glucose in the intestine tends to inhibit hunger activity and does not stimulate the pancreas, we made extensive use of 5 per cent glucose as a control solution, especially in perfusion experiments.

Discussion. The pH threshold for pancreatic stimulation by means of acid in the intestine appeared to vary between pH 3.0 and pH 5.0 depending on the acid used, but the variations are of doubtful significance. They appear to be fully explained by the demonstrated differences in the ability of the solutions to maintain their acidity in the intestine. It is probably safe to assume that the true threshold is reasonably constant and could be measured accurately if a constant pH could be maintained at the point of contact of the stimulus with the reacting mechanism. A nearer approach to this ideal condition is obtained with the stronger buffers such as citrate or glutamic acid than with the weaker ones such as HCl or phosphate; therefore, the true threshold is probably not below pH 5.0, which was the apparent threshold for the best buffer.

It does not necessarily follow that acidity will be an effective stimulus for the pancreas when the intestinal contents during digestion are no more acid than pH 5.0. The results obtained by the perfusion method show that even when an acid solution in the intestine is continuously renewed at a constant pH the buffering capacity as well as the acidity of the solution is a factor in determining its effectiveness as a stimulus. The buffering of the intestinal contents will, doubtless, vary considerably under different conditions. In one experiment on a dog following a meal of raw meat the

intestinal contents were found to have slightly less buffering capacity than a 0.15 M lactic acid solution (fig. 3). Under these conditions no stimulation due to acid could be expected at pH 5.0 and only a barely perceptible amount at pH 4.5. The practical threshold for bringing about pancreatic secretion at a rate comparable to that normally present during digestion would probably be in the neighborhood of pH 4.0.

Thomas (1940) found that in dogs fed raw meat the contents of the first part of the duodenum varied in acidity between pH 2.4 and 7.0 but were generally near pH 4.0. Therefore, under some physiological conditions the acidity of the intestinal contents during digestion is adequate to stimulate the pancreas.

The fact that the practical threshold for pancreatic stimulation by means of acid and the prevailing acidity of the duodenal contents under the same conditions are alike (pH 4.0) indicates that one function of the acid mechanism is to control intestinal acidity.

SUMMARY AND CONCLUSIONS

1. A method is described for the study of pancreatic function without a pancreatic fistula.
2. An attempt was made to determine the pH at which acid in the intestine begins to act as a stimulus for pancreatic secretion.
3. Variations in pH threshold were observed but these were interpreted as due to differences in the ability of buffer solutions prepared with different acids to maintain their acidity in the intestine.
4. The pH threshold for the more efficient buffers was near pH 5.0. We conclude that the true threshold, if it could be determined, would not be below pH 5.0.
5. The practical acid threshold for causing a significant amount of secretion in the dog digesting raw meat was estimated to be near pH 4.0. This degree of acidity is commonly present in the dog's duodenum during meat digestion.
6. We conclude that under some physiological conditions the acidity of the intestinal contents is adequate to stimulate the pancreas.

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SOME FACTORS AFFECTING THE SPECIFIC DYNAMIC ACTION OF FAT IN NORMAL AND PARTIALLY DEPANCREATIZED RATS

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The elevation of basal metabolism which follows pancreatectomy in dogs and cats has at times been ascribed to the increased activity of the thyroid gland or of the adrenal medulla. Ring and Hampel (1933a) brought evidence to show that a large metabolic increase may occur following the removal of the pancreas in animals lacking the thyroid gland or functional adrenal medulla. Furthermore, the increased protein metabolism of diabetes in our opinion fails to account for the stimulation of metabolism (see Ring and Hampel, 1933b; Ring, 1936). In 1935 Ring showed that in partially depancreatized cats if the basal metabolism is approximately normal, the specific dynamic action of fat is elevated. On the other hand, in more severe diabetes, associated with a high basal metabolism, the calorigenic action of fat disappears. These facts suggest that the processing of fat is greater in diabetes than in the normal state and in fact may be so rapid that the ingestion of fat cannot further increase this processing.

Method. For continuing this work we have chosen rats so that the observations could be made on a larger and more homogeneous group. Only animals which would remain quiet for long periods of time were selected. The metabolism was measured by using a modification of Benedict's "Multiple Chamber Respiration Apparatus" which recorded continuously the oxygen consumption of eight rats kept in separate jars. The graphs obtained made it possible to measure the oxygen consumed during periods when the animals were quiet. No determinations of carbon dioxide production were made as previous studies had shown that the respiratory quotients were changed not more than 0.01 or 0.02 as a result of fat ingestion. The estimation of heat production was based on the assumption of a respiratory quotient of 0.72. The surface area formula used was that devised by Lee (1929).

In the first group of experiments, rats were fasted for fifteen hours then placed in the respiration apparatus where the oxygen consumption was recorded continuously for eight hours. Quiet periods each hour were

measured and averaged to obtain the figure for basal metabolism. A week later, the same animals, again postabsorptive, were given 1.5 cc. of oleic acid each by mouth just before beginning the eight hour period of metabolic measurements. These experiments were alternately repeated

TABLE 1

Calorigenic effect of oleic acid in normal rats and depancreatized rats

RAT	BASAL MET. (CAL. PER SQ.M. PER DAY)	INCREASE AFTER OLEIC ACID		BASAL MET.	INCREASE AFTER OLEIC ACID	
		Cal.*	Per cent		Cal.*	Per cent
			Normal			
A	778	59	7.5			
B	787	67	8.5			
C	788	35	4.4			
D	758	20	2.6			
E	789	47	6.0			
F	806	45	5.6			
G	854	52	6.1			
H	807	46	5.7			
I	845	84	10.0			
J	844	60	7.1			
K	844	71	8.4			
Average increase.......		53.3	6.6			
			Depancreatized			
					After fat diet	
1	721	67	9.3			
2	736	70	9.5			
3	769	48	6.2			
4	746	55	7.4			
5	693	57	8.2			
6	741	60	8.1			
7	665	58	8.0	721	123	17.0
8	762	57	7.5	735	120	16.3
9	736	54	7.3	845	114	13.5
10	636	54	8.5	840	79	9.4
11	727	75	10.3	844	90	10.6
Average increase.......		59.5	8.2		105.5	13.4

* Increase, given in calories per square meter per day, was determined during a period of eight hours.

during subsequent weeks until there were at least four basal figures and three determinations following the ingestion of oleic acid. It was later found that this procedure could be shortened without reducing the accuracy of results by simply measuring the basal metabolism for the three hours just prior to the ingestion of oleic acid.

In depancreatizing the rats, the operative procedure of Shapiro and Pincus (1936) has been followed. The metabolic studies were begun when these animals had reached a weight of 150 grams or more.

Since numerous observations have shown that the adrenal cortex may play a rôle in the development of typical pancreatic diabetes (see Hartman and Brownell, 1934; Lukens and Dohan, 1938), it is of interest to know how cortical extract affects the specific dynamic action of fat. The Wilson Laboratories kindly supplied us with their preparation. Similar results were obtained using extract prepared in this laboratory. Five cubic

TABLE 2

The specific dynamic action of fat before and after the ingestion of a fat diet for three days

RAT	BEFORE FAT DIET		AFTER FAT DIET	
	Cal.*	Per cent increase	Cal.*	Per cent increase
		Normal		
L	59	7.9	73	9.2
M	67	8.0	69	8.2
N	13	1.7	52	6.3
O	56	7.7	87	11.2
P	68	8.5	58	7.2
Q	57	7.5		
Average.....	53.3	6.9	67.8	8.4
		Depancreatized		
19	56	7.7	93	15.0
12	33	4.7	133	19.4
15	73	9.3	112	14.1
16	45	6.3	85	13.6
17	77	10.8	109	15.5
Average.....	56.8	7.8	106.4	15.5

* Increase given in calories per square meter per day was determined during an eight-hour period after the ingestion of fat.

centimeters of the extract were given intraperitoneally prior to the basal metabolism measurements.

RESULTS. In the left-hand columns of table 1 will be found the averages of results on normal animals using the first procedure (basal metabolism measured for an entire day). Table 2 contains the results using a three-hour preliminary basal measurement. It will be seen that the figures are quite similar using either method. The partially depancreatized rats show almost the same S.D.A. as the normal rats if they are kept on their regular diet (Purina Dog Chow). This, we believe, is because the diabetes produced in the rat by pancreatectomy is very mild. When the operated

animals are placed on an exclusive fat diet (olive oil) for three days in order to increase the severity of the diabetes, then changes are produced which bring about a decided increase in the specific dynamic action of fat during the subsequent weekly measurement even though these rats are eating their regular diet once more (see tables 1 and 2). Normal animals treated in the same way show negligible changes. That the differences found are not due to poor nutritional state of the operated animals, is shown by their

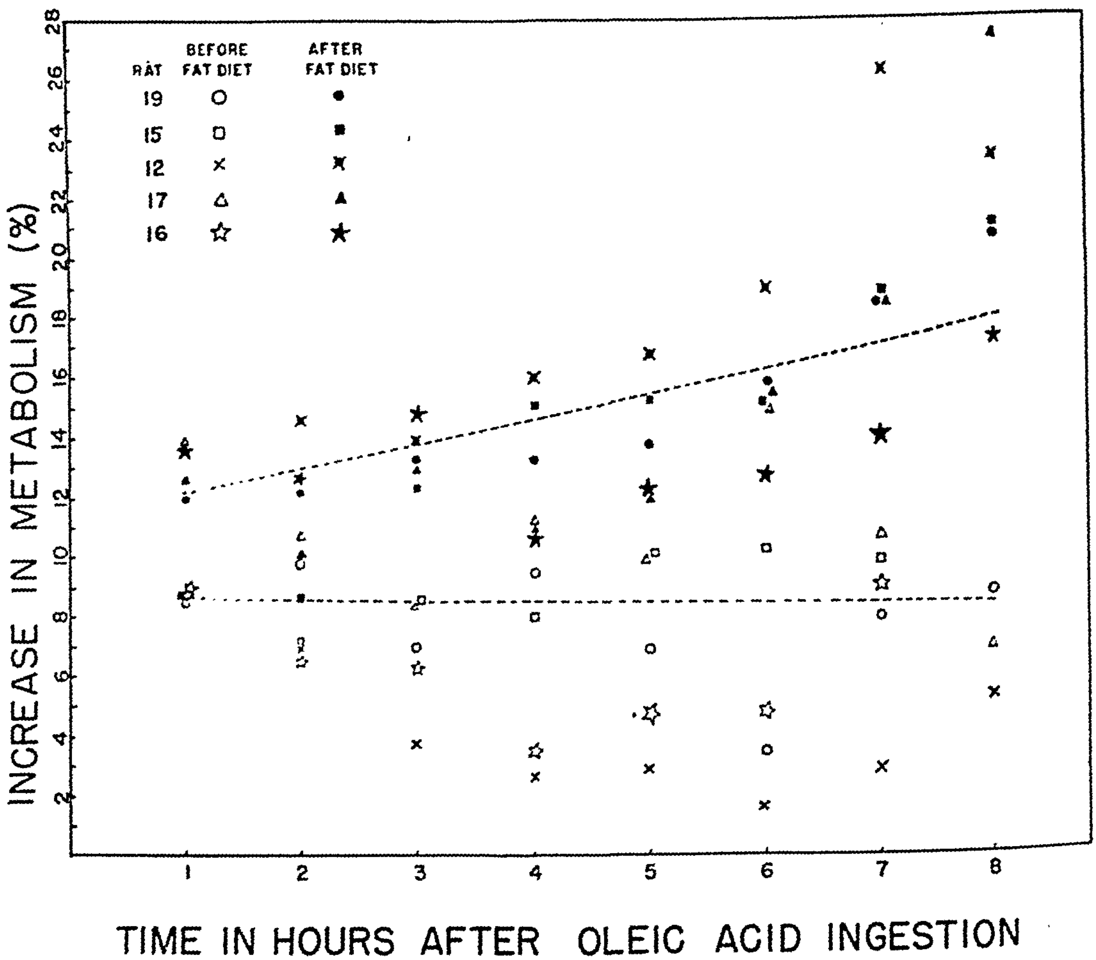

Fig. 1

appearance and also by the determination of the nutritional correction factor of Cowgill and Drabkin $\frac{\text{Weight}^{\frac{1}{3}}}{\text{Body Length}}$. In five normal animals measured, this factor varied between 0.270 and 0.287. Seven operated animals gave figures between 0.271 and 0.302.

Although all animals appeared to be very quiet for a large part of the time they remained in the respiration chamber, it seemed possible that

activity was not properly recorded. In order to check this, three depancreatized and four normal rats were given water instead of oleic acid. The following are the average changes in metabolism of each rat during the next eight hours: +1.3 per cent, +1.2 per cent, +0.4 per cent, +0.3 per cent, −0.5 per cent, −0.7 per cent.

The course of the metabolic changes in the depancreatized rats during the eight-hour period after fat ingestion is plotted in figure 1. The results on normal animals if given here would duplicate those shown for depancreatized rats before the latter were placed on an exclusive fat diet. There is a slight elevation of metabolism which is maintained throughout the period of measurement. The failure of the metabolism to return to the basal level by the end of eight hours is not surprising since about six hours are required to complete the absorption of the fat ingested (see Barnes, Wick, Miller and MacKay, 1939) and the calorigenic effect must last for some time after this. A few of our experiments suggest that as large a calorigenic effect is obtained with a smaller dose of oleic acid (1 cc.). After the depancreatized rats have been kept on the fat diet for three days, the S.D.A. during subsequent weekly measurements is larger and the metabolism may continue to rise throughout the eight hour period. Three animals studied eighteen hours after fat ingestion still showed an increase above basal metabolism of 12 per cent, 15 per cent and 16 per cent.

TABLE 3

Effects of cortin on the calorigenic action of fat

NORMAL			DEPANCREATIZED		
Rat	Control*	After cortin†	Rat	Control*	After cortin†
L	61	8	19	94	61
M	73	11	16	78	40
O	48	24	15	96	45
N	67	42	12	110	22
Q	56	00	17	81	59
Average......	61	19		92	45

* Average of four measurements of calorigenic action of fat in weeks preceding and following cortin injection, given in calories per square meter per day.

† Average of at least two determinations.

The effect of cortical extract on the calorigenic effect of fat is shown in table 3. It will be seen that the S.D.A. is depressed in both normal and depancreatized rats. It is believed that the S.D.A. of fat is due to the processing of this material in the liver. This processing is, no doubt, less when there are sizable glycogen stores. Many have shown that liver glycogen is increased after the injection of cortical extracts (see Russell,

1940, and this may well explain the results which were obtained. The diabetic animal with less store of carbohydrate, processes more fat and the S.D.A. is not depressed to as low a level by extract.

CONCLUSIONS

1. In rats, the specific dynamic action of fat is not affected by pancreatectomy unless the animals are on a fat diet for at least three days. Then the calorigenic action of fat in the operated animals is approximately doubled. The fat diet does not greatly affect the S.D.A. in normal animals (see tables 1 and 2).

2. Since the partially depancreatized rats studied were not undernourished, nutritional condition cannot be responsible for the results obtained.

3. Giving large doses of cortical extract depresses both the S.D.A. of fat of normal and depancreatized rats (see table 3).

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THE RELATIONSHIP BETWEEN SALT INTAKE AND THE POLYURIA OF EXPERIMENTAL DIABETES INSIPIDUS

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Functional deficiency of the posterior lobe of the pituitary is characterized not only by an augmentation of the water exchange, but also by a disturbance in the salt metabolism. Evidence that diabetes insipidus is accompanied by some derangement of the salt regulating mechanism includes the following observations: there is a delay in the excretion of salt when it is ingested in large amounts by diabetes insipidus patients (1), there is a relative inability of the kidney to concentrate salt (2, 3), and animals with diabetes insipidus do not show the changes in serum sodium and chloride following adrenalectomy which are characteristic of non-polyuric animals (4).

More direct evidence of a definite relationship between salt metabolism and diabetes insipidus is seen in the work of Swann (5, 6, 7). Working with hypophysectomized rats, Swann concluded that the polydipsia of diabetes insipidus is secondary to the disturbance in salt regulation. He ascribed the loss of polydipsia which occurs during fasting to the absence of salt intake in the fasted animal, for he found that in hypophysectomized rats the polydipsia was markedly reduced on a diet nearly salt-free, but persistent if salt was given to a fasting animal. This work leads to the view that with low salt intake there would be no polyuria or polydipsia; with moderate salt intake, a moderate polyuria; with high salt intake, a high degree of polyuria. If these findings on the hypophysectomized rat can be shown to be of general application, then they are of very great importance, because diabetes insipidus then becomes mainly a disturbance in sodium chloride metabolism, and in order to control the polyuria and polydipsia it would be necessary only to limit the salt intake of an individual suffering from this disease. We have, therefore, sought to obtain information concerning the relationship which may exist between salt intake and the polyuria of diabetes insipidus, using as test animal the cat with interrupted supraoptico-hypophyseal tracts. The general methods for production of the experimental diabetes insipidus (d.i.) and for care of the animals have been mentioned elsewhere (4).

OBSERVATIONS. Table 1 shows that a close parallelism can be demon-

strated between salt intake and urine output in diabetes insipidus, when the NaCl intake is on a high level. The cat shown in the table, a typical example of several experiments, received 1.5 grams of sodium chloride daily added to his food, and in addition he was given 0.5 per cent NaCl solution to drink. This solution was kept continuously in front of the animal instead of water, and the intake was voluntary. Normal animals could not be induced to take in such large quantities of salt.

TABLE 1

Effect on urine volume of adding 1.5 grams of NaCl to meat and substituting 0.5 per cent NaCl solution for drinking water, in a cat with diabetes insipidus

NORMAL DIET			HIGH SALT REGIMEN		
Date	NaCl intake	Urine volume	Date	NaCl intake	Urine volume
	grams	*cc.*		*grams*	*cc.*
1/21	0.238	460	1/25	2.738	575
22	0.238	470	26	4.988	750
23	0.238	525	27	3.238	640
24	0.238	415	28	6.488	1225
			29	12.238	1920
			30	9.988	1660
			31	12.738	2160

TABLE 2

Effect on the urine volume of adding one to two grams of salt to the daily diet

KIND OF ANIMALS	NUMBER OF ANIMALS	DAILY AVERAGES						
		Normal diet			High salt diet			
		Urine volume	NaCl intake	Days	Urine volume	NaCl intake	Days	Increase in urine volume on high salt
		cc.	*grams*		*cc.*	*grams*		*cc.*
Normal	2	119	0.321	12-14	137	1.238	6-7	18
	5	127	0.271	12-15	170	2.158	6-36	43
Diabetes insipidus	3	569	0.238	5-13	760	1.240	10-41	191
	2	705	0.238	10-13	973	2.120	14-17	268

Table 2 shows the difference between the responses of normal and of d.i. cats to the addition of a moderate amount of sodium chloride in the diet, while the animals are allowed water ad libitum. One to two grams of extra salt daily makes very little difference in the urine volume of normal cats, but greatly exaggerates a polyuria already present in the d.i. animals. This confirms some of Swann's observations, as well as the results of similar experiments by Fisher, Ingram and Ranson (8). The effect

of ingested salt in increasing the polyuria of diabetes insipidus is undoubtedly related to the relative difficulty with which d.i. animals concentrate salt in the urine.

Although it is apparent from the results of the salt-feeding experiments that there is a positive relationship between salt intake and the degree of the polyuria when the salt intake is high, it does not follow that the polyuria ordinarily observed in diabetes insipidus is likewise related to the salt which is contained in the ordinary diet.

Table 3 gives a protocol of a cat which was placed on a diet almost entirely free of chloride. This was our regular cat ration of ground beef, with the salt extracted by boiling distilled water according to the method described by Swingle et al. (9). This "salt-free" diet seems to be rather unpalatable, and after a variable number of days the cats refuse it, and go

TABLE 3

Effect of "salt-free" diet and of fasting on the polyuria of a cat with diabetes insipidus

DATE	NaCl INTAKE	URINE VOLUME	URINE SP. GR.	REMARKS	DATE	NaCl INTAKE	URINE VOLUME	URINE SP. GR.	REMARKS
	grams	*cc.*				*grams*	*cc.*		
8/10	0.238	340	1.006	Regular	8/19	0.243	355	1.008	"Salt-free" diet,
11	0.238	340	1.010	diet	20	0.183	460	1.010	with NaCl
12	0.238	365	1.009						added
13	0.238	380	1.006		21	0	95	1.009	Fasted, water ad
14	0.238	260	1.005		22	0	70	1.015	lib
					23	0	135	1.012	
15	0.005	460	1.009	"Salt-	24	0.212	210	1.004	Regular diet
16	0.005	370	1.007	free"	25	0.238	300	1.010	
17	0.005	435	1.008	diet	26	0.238	400	1.009	
18	0.005	355	1.011		27	0.238	450	1.005	

into a voluntary fast. It will be noted from the table that although the diet is almost completely free of chloride, the animals maintain their polyuria so long as they eat it, and the addition of salt in the amount present in the normal daily food intake does not affect the polyuria. On the very first day of fast, however, the urine volume drops to a level nearly corresponding to that which is characteristic of the normal animal. Since the polyuria was maintained on a diet practically free of salt, it was evidently not the lack of salt which caused the reduction in the urine volume when the fast started. The figures for the urine specific gravity were throughout the period at a level characteristic of the d.i. cat, even on the days of fast; so, judged by this criterion, the animal still had diabetes insipidus even on the days when there was no obvious polyuria. Similar experiments were performed on four cats altogether, but as the other re-

sults were fully confirmatory of those shown, they are omitted from the table.

Another experiment on a fasting d.i. cat is summarized in figure 1. In the first fasting period shown, the fast was complete except for water, which was freely available. It may be noted that during this period there was a prompt reduction in urine volume. The second fasting period was the same as the first, except that the salt intake was kept constant by the addition of a small amount of salt to the drinking water. Yet, in spite of the constant salt intake, the drop in urine volume was as marked and as prompt as before. Comparable experiments performed on two other animals gave fully concordant results.

On the basis of the experiments summarized above, we conclude that the d.i. cat does not react to these experimental conditions in the same way as has been reported for the hypophysectomized rat. The decrease in

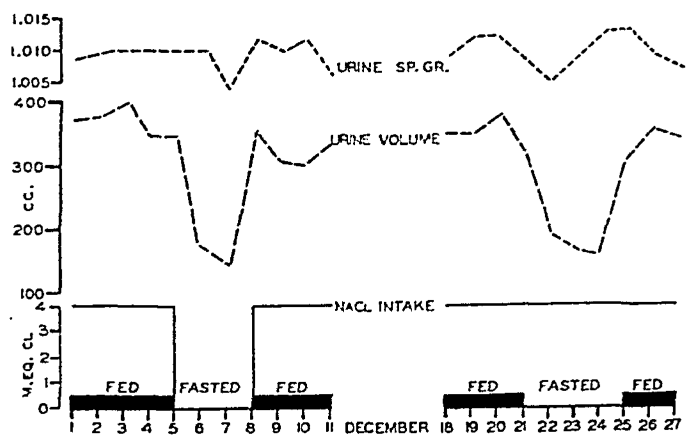

Fig. 1. For explanation see text

urine volume observed during the fasting of d.i. cats is not due to the salt deprivation which accompanies fasting, nor is the polyuria of diabetes insipidus solely dependent on the salt content of the normal diet. If there is any element in the diet which is responsible for the polyuria, it is not the salt alone.

SUMMARY

Cats with diabetes insipidus produced by interruption of the supraoptico-hypophyseal tracts show a close parallelism between salt intake and urine volume when the NaCl intake is on an abnormally high level, but this parallelism does not hold for salt intakes of normal or below normal levels.

During fasting, the urine volume of a diabetes insipidus cat falls almost to the level which is characteristic of a normal animal. The loss of the polyuria is not due, however, to the salt deprivation which accompanies fasting, because if the salt intake is kept at a normal level during the

fast by adding salt to the drinking water, the urine volume falls just the same. Furthermore, the animal maintains his polyuria practically intact when placed on a diet almost completely free of salt.

It is evident, therefore, that if there is any factor in the ordinary diet which causes the maintenance of the large fluid exchange of diabetes insipidus, it is not the sodium chloride alone which is responsible. We conclude that the claim which has been made for the hypophysectomized rat, that the polyuria is dependent on the salt in the ordinary diet, can not be valid for the d.i. cat.

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THE EFFECT OF DISTENTION ON BLOOD FLOW THROUGH THE INTESTINE

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It would be expected that any increase in extra-capillary pressure would increase the resistance to blood flow by narrowing the smallest vessels. The behavior of the pulmonary bed conforms to this expectation (De Jager, 1885). The apparent lack of conformity of the intracranial bed has not yet been adequately explained (Ferris, 1939). There is no question that a rise in intra-enteric pressure from whatever cause elevates extra-capillary pressure in the walls of the intestine. A passive rise in intra-enteric pressure might be expected to interfere with blood flow somewhat less than a rise due to muscular activity, since during distention the sub-mucosa and inner muscle layers probably to some extent protect the more peripheral layers (Mall, 1896). This may, however, be offset by angulation of vessels in the over-distended intestine (Eisburg, 1925). It is doubtful, therefore, if data relating blood flow to the pressure developed within the intestine by muscular activity (Anrep, Cerqua and Samaan, 1934) can be used in predicting the effect on flow of pressures developed within the intestine by inflation.

Van Zwalenburg (1907) observed stoppage of flow in a few capillaries in the dog's intestine when intra-enteric pressure was raised by inflation to 30 mm. Hg. No data were given on the effect of lower pressures. Gatch and Culbertson (1925), Gatch, Trusler and Ayers (1927), and Dragstedt, Lang and Millet (1929) reported a reduction in venous outflow from the distended intestine of the dog proportional to the elevation of lumen pressure. In preliminary experiments (Lawson and Chumley, 1940) we failed to obtain complete confirmation of these observations, since we found no sustained reduction in blood flow with pressures below a critical level which was usually about 30 mm. Hg. The present report extends these observations, and demonstrates the existence of intrinsic compensating mechanisms through whose operation the flow of blood through the intestine may be capable of being maintained without reduction in the face of elevated lumen pressure.

Methods. Loops of ileum 6 to 12 cm. long were isolated between ligatures in barbitalized dogs. Arterial flow into the loop was measured

by differential manometry, using a modification of the technique described by Lawson and Holt (1939). The superior mesenteric artery was dissected out for 1 to 2 cm. above and below the origin of the branch supplying the loop, and the constricting clamp was placed on the artery central to the branch. A second branch, below the constriction, was cannulated and connected with one limb of the differential manometer, and all other branches below the constriction were ligatured. The carotid, the femoral, or a convenient branch of the superior mesenteric artery above the constriction was cannulated and connected with the other limb of the manometer. A mercury manometer was connected through a T in this line to give an absolute reading of systemic arterial pressure.

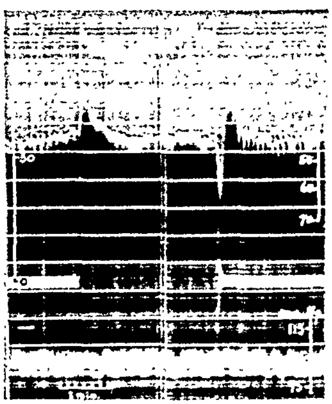
A differential metal bellows manometer was used for recording the fall in pressure across the constriction in most of the experiments (Lawson, 1940). This system, to give satisfactory records, requires a lowering of pressure in the artery below the constriction of 10 mm. Hg or more. Since the level of pressure in the intestinal arteries is undoubtedly a factor in determining the effect of distention on blood flow, an inverted air-water manometer of the type described by Wagoner and Livingston (1928) was used in a portion of the work for comparison. The central limb of the manometer was expanded to form a well, and optical records were made of the changes in fluid level in the peripheral limb. With this type of manometer satisfactory records were obtained with mean pressure in the intestinal arteries (below the constriction) only 0.5 to 3.5 mm. Hg below mean systemic arterial pressure. Results with the two types of manometer were qualitatively similar. No quantitative comparison was attempted.

Calibration of the flow-meter to obtain data in terms of volume flow was not done routinely. Since slight changes in the tonus of the artery or deposition of fibrin at the constriction may change the calibration of the flow-meter, the calibration to be valid should be repeated at intervals during the experiment. As this requires shutting off the artery supplying the loop and opening a second artery below the constriction for collecting blood, it entails some damage to the intestine, and was usually not done. The records show therefore only the pressure difference across the constriction. Assuming that the dimensions of the constriction and the viscosity of blood remain constant over the short periods occupied by each record, an increase in the pressure difference means an increase in blood flow, and *vice versa* (Lawson and Holt, 1939). Without proof that the calibration of the flow-meter has remained constant, the records cannot be used for comparing the amount of flow in widely separated portions of the same experiment.

In the preliminary studies the loop of intestine was distended by inflating with air a large condom balloon lying within the lumen against a water or mercury manometer. In the later experiments pressure at any

level of distention was kept constant by inflating either the balloon or the loop directly with water from a leveling bottle. No inflations above 60 mm. Hg were studied. It is doubtful that pressure within the dog's small intestine rises above 40 mm. Hg even in experimental intestinal obstruction (Antoncic and Lawson, 1940). The effect of distention prolonged beyond 5 minutes was not studied.

RESULTS. Typical changes in blood flow during and following a period of inflation are shown in figures 1 and 2. For descriptive purposes five phases in the response may be recognized.



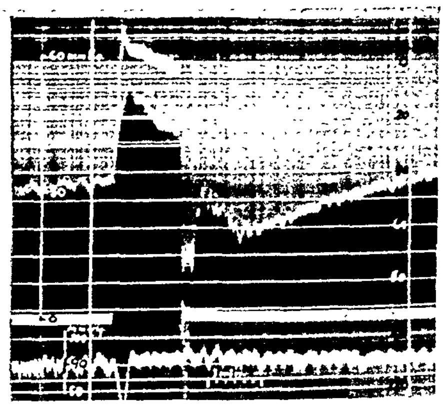


Fig. 1 (left). Records from above down are: 1, pressure difference in mm. H_2O between carotid and mesenteric arteries across constricting clamp, recorded optically from air-water manometer (see text). An ascending record indicates a fall, and a descending record a rise in the pressure difference. 2, pressure within loop of ileum, inflated during the period of sudden elevation against a water manometer. 3, carotid arterial pressure recorded from a mercury manometer. The response to inflation is typical except for the absence of phase 5 (see text).

Fig. 2 (right). Construction of figure as in figure 1. The record shows exaggerated post-inflation hyperemia (phases 3–5, see text) following brief, moderately high pressure inflation.

Phase 1. Flow was reduced at the moment of inflation, usually reaching its greatest reduction within 8 to 10 seconds after the inflation was complete.

Phase 2. There was always a tendency for flow to recover its control value during sustained inflation, even though lumen pressure was kept constant by distending the intestine from a leveling bottle rather than against a manometer (fig. 4). The rate of recovery during this phase was usually rapid during the first 10 to 15 seconds, then slower until a plateau was reached within 1 to 3 minutes of the inflation. Usually with distending pressures below 30 mm. Hg (fig. 6) and sometimes with pressures up to 60 mm. Hg (figs. 1, 4) the recovery of flow during this phase was complete, flow during the remainder of the distention period continuing at control levels.

Phase 3. On deflation there was typically a sudden increase in flow over control levels, the peak of the increase being reached at the time of complete deflation. With brief or low pressure inflations this phase usually terminated the response, flow gradually declining to its control value within 5 to 90 seconds (fig. 6, 1st inflation). The hyperemia of phase 3 was observed even though flow had fully recovered its control value during phase 2 (fig. 6, 1st distention) and in animals in which distention caused an increase in flow (fig. 5).

Phases 4 and 5. With longer or larger distentions the period of post-inflation hyperemia was prolonged, the greatest duration observed in our studies being 6 minutes. Under these conditions the hyperemia was usually interrupted within 15 to 30 seconds of its beginning by a period of reduced flow lasting 10 to 50 seconds (phase 4) during which flow was

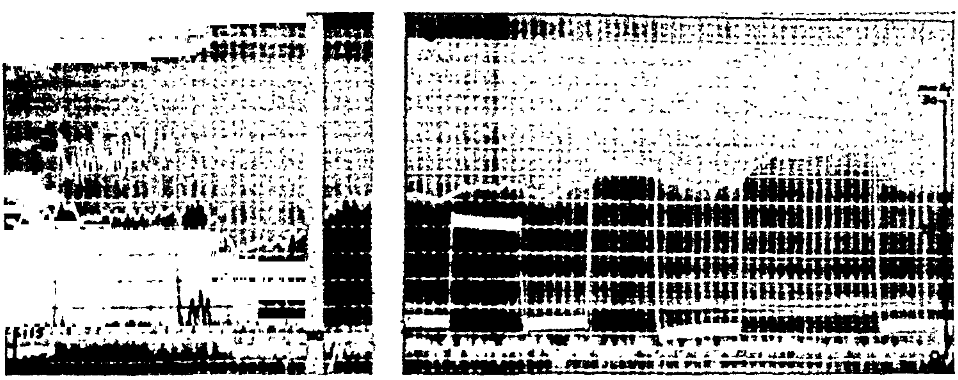


Fig. 3. Construction of figure as in figure 1. Time in 10-second intervals. A (left), effect of inflation on the freely distended loop, before application of plaster cast. Air trapped in balloon on deflation records hypermotility during phase 4 of the response (see text). B (right), effect of three inflations of varying degree after application of plaster cast.

sometimes less than during the control period (fig. 2). Records of intestinal muscular activity made by incompletely deflating the gut showed intense motor activity at this time (fig. 3 A). Rhythmic variations in flow, synchronous with the beat of the intestine, with flow at its minimum value at the peak of each gut contraction, were often observed. The fifth phase consists of a resumption of hyperemic flow levels following the termination of phase 4, with gradual return to the control (fig. 2).

The response to pressure without distention. It would be expected that an initially reduced blood flow would decline still further as the intestine distends, and as pressure in the outer layers of the gut wall rises. Since, instead, flow always increased during phase 2 as the intestine enlarged, it was decided to prevent the enlargement of the loop by encasing it in plaster-of-Paris.

A comparison of the response of the same loop before and after ap-

plication of the cast showed striking differences (fig. 3). In every case the jacketed loop showed a simple monophasic reduction in flow, persisting throughout the period of inflation, with no tendency to return toward the control. On deflation from moderate inflation levels (below 40 mm. Hg) the control flow was immediately resumed, with no suggestion of any part of the post-inflation period of hyperemia (phases 3–5) typical of the dis-

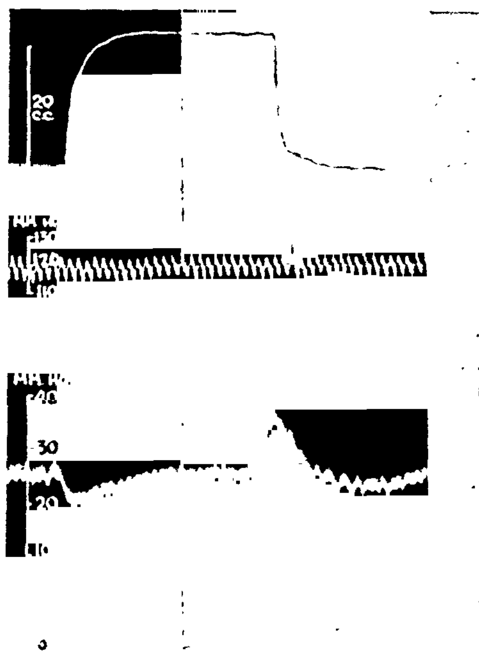


Fig. 4 (left). Records from above down are: 1, volume of water accepted by loop of ileum distended from leveling bottle at height of 54 cm. H_2O (40 mm. Hg). Record made with volume recorder connected above water level in leveling bottle; 2, carotid arterial pressure; 3, pressure difference in millimeters of mercury between carotid and mesenteric arteries across constricting clamp, recorded with differential metal bellows manometer. An ascending record indicates a rise, and a descending record a fall in the pressure difference. Time in intervals of 10 seconds and 1 minute. A reference ordinate has been erected at the point where the pressure difference levels off at its control value, approximately 20 seconds after full enlargement of the loop.

Fig. 5 (right). Construction of figure as in figure 4, except that the two uppermost records are in inverse order. To show increased blood flow through loop distended under pressure of 23 mm. Hg. Note well-marked phase 3, and slight phase 5 on deflation (see text).

tended loop. That the jacketed loops were capable of reactive hyperemia was proven by clamping the artery for 90 seconds or more. Hyperemia was also elicited following inflations above 80 mm. Hg lasting 2 to 3 minutes.

The response to stretch. Since a comparison of the freely enlarging with the encased loop demonstrates that enlargement of the intestine is in some way responsible for the maintained blood flow during inflation, it should

be possible to stretch the intestine in such a way as occasionally to produce a pure increase in flow. In the hope that the compensating mechanism might be set in operation without excessive increase in extravascular pressure in the gut wall, loops were laid open along their antimesenteric border, the cut edges placed in bulldog paper clips, and stretched transversely by hand. In one animal flow was increased during stretch on every trial. In three animals an initial increase in flow progressively deteriorated, and at the end of approximately one hour flow was consistently reduced by

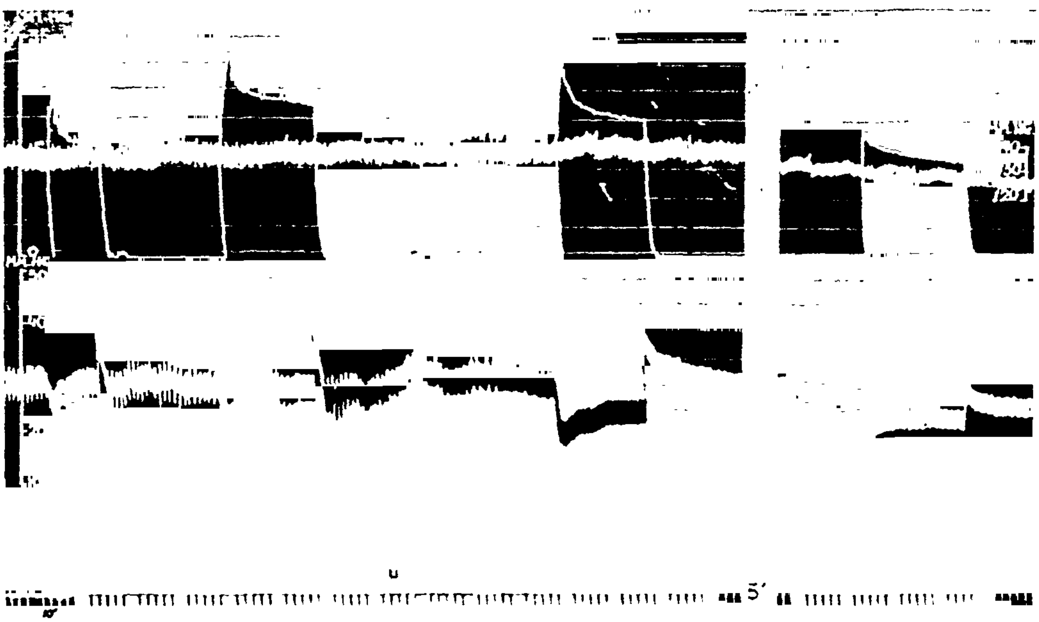


Fig. 6. Construction of figure as in figure 4, except that second record from top is a manometer tracing of inflation pressure. There is complete restoration of blood flow during the first inflation (initial pressure approximately 25 mm. Hg) and nearly complete restoration during the second (initial pressure approximately 35 mm. Hg). During the third inflation to approximately the same height as the second, but following injection of 1 per cent cocaine hydrochloride into the lumen of the loop at signal, there is less complete restoration of flow. There is almost no restoration of flow, and almost no post-inflation hyperemia following the fourth inflation, smaller than either of the first two.

stretch. In the remaining animal, a decrease in flow was obtained initially. After local application of 0.1 per cent pilocarpine, stretch caused an increase in flow, which was abolished after local application of 0.1 per cent atropine (fig. 7).

The effect of decentralization on the compensating mechanism. Perivascular section of the mesenteric nerves was without immediate effect on any phase of the response to distention. In two animals, however, whose loops had been decentralized an hour or more before starting the experiment

and in which the intestine was active and in high tonus, all distentions below 50 mm. Hg produced an increase in flow over the control during the inflation period (fig. 5). A similar increase was obtained in two other animals in our series, without denervation. In all these, the post-inflation behavior (phases 3–5) resembled that of loops whose flow was reduced during distention.

The effect of local anesthetics on the compensating mechanism. Compensation during phase 2 was either wholly abolished, or greatly reduced by application of 1 per cent cocaine hydrochloride to the mucosa of the loop (fig. 6). In every case the maximum distention pressure for which full

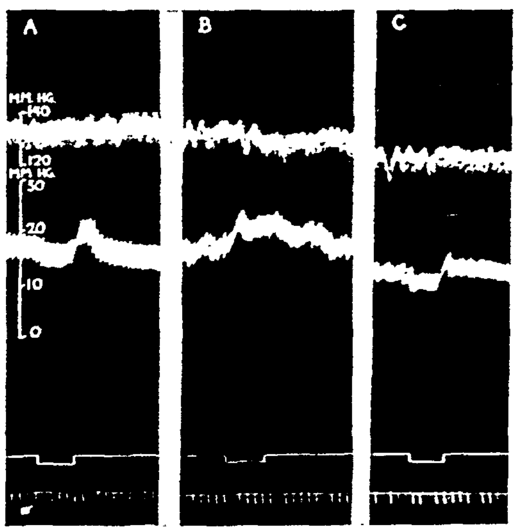


Fig. 7. Records from above down are: 1, carotid arterial pressure; 2, pressure difference between carotid and mesenteric arteries as in figure 4. The signals mark the application of transverse stretch to the loop of ileum (see text for method). In each trial the loop was stretched to a measured width of 4 cm., regardless of tonus or control width. A, initial response of untreated loop. B, after application of 0.1 per cent pilocarpine nitrate to the mucosa of the loop. C, after application of 0.1 per cent atropine sulfate to the mucosa.

compensation could be effected was markedly lowered after cocaine. Post-inflation hyperemia following moderate distentions was either greatly reduced or entirely lacking in cocainized loops, although these loops responded to temporary arterial occlusion with hyperemia on restoration of flow. The response of the fully cocainized loop thus closely resembled the response of the loop encased in plaster-of-Paris.

There was also a reduction of the maximum pressure permitting full compensation in loops treated with 1 per cent procaine hydrochloride, although complete abolition of compensation during phase 2 was never attained. There was not as complete suppression of the post-inflation hyperemia as after cocaine.

DISCUSSION. The over-reduction of flow at the moment of inflation (phase 1) and the over-increase of flow at the moment of deflation (phase 3) were tentatively interpreted in the earlier communication as the result of pressure changes in the capillary area due to capacity alterations on compression and decompression (Lawson and Chumley, 1940). This interpretation was supported by the demonstration of simultaneous changes in the opposite direction in venous outflow at these times. The absence of these phases in the response of the intestine encased in plaster or treated with cocaine necessitates a re-interpretation, since these procedures would not abolish such capacity changes. That during inflation blood is expressed from the distending gut at the same time that arterial inflow is most sharply reduced, and that during deflation blood pools in the gut during the time that arterial inflow is greatest, is clear from the earlier data. But these demonstrated changes in the volume of blood pooled in the intestine and the attendant changes in the pressure gradient do not appear to affect the resistance to arterial flow. It is possible that they occur for the most part in the veins.

These phases in the response assume new significance in the light of the present demonstration that a compensating mechanism is set in operation by distention of the intestine. Phase 1 probably represents the mechanical reduction in flow due to the increased extravascular pressure, since the flow level reached during this phase remains constant if the compensating mechanism is silenced by encasing the gut or treating it with cocaine. Phase 3 probably represents, in part at least, the persistent action of the compensating mechanism after extravascular pressure has returned to normal, since it is not seen unless some compensation has been elicited. This interpretation of phase 3 is supported by the observation that the increased flow set up by transverse stretch of the intestine only slowly declines after cessation of stretch (see fig. 7B).

In the earlier report a portion at least of the post-inflation hyperemia was tentatively identified as reactive hyperemia. Both the cocainized intestine and the intestine encased in plaster are capable of reactive hyperemia following complete arterial occlusion or excessive inflation. They do not, however, appear to repay the flow deficit incurred during moderate inflations, even though this is greater than the deficit incurred in the untreated, freely distended gut. The part played by reactive hyperemia in the post-inflation behavior of the freely distended untreated intestine is therefore not clear, and will require a quantitative study of reactive hyperemia in the intestine for elucidation.

The site of the compensation cannot be determined from these data. Dragstedt, Lang and Millet (1929) observed a residual flow of blood from the mesenteric veins which no amount of intra-enteric pressure could stop. This was interpreted as passing through mesenteric anastomotic channels.

They reported that this flow gradually increased during prolonged distention until it nearly equaled the original, presumably due to dilatation in the mesenteric channels. Their observations appear to have been made with intra-enteric pressure well above mean arterial pressure, to ensure that no blood could flow through the gut walls. We have not repeated this work. It does not appear likely, however, that we have elicited this phenomenon, since in our experiments flow reaches a steady state short of full compensation with distentions above a certain critical level, but well below the level of mean arterial pressure. Our data show only that total flow through the system intestine-mesentery returns to normal during distentions below the critical level, and offer no evidence against drastic redistributions of flow within this system.

The data offer more valid evidence regarding the nature of the compensating mechanism. It is clear that stretch of the intestinal walls either mechanically reduces the resistance to flow, or sets in operation a resistance-lowering mechanism. Acceptance of the first alternative would have to be based on the assumption that in the undistended intestine resistance to blood flow is kept high by angulation and tortuosity of vessels. Were this the case, it would be expected that restoration of flow during the phase of compensation would closely parallel the enlargement of the intestine. Our records fail to support this interpretation, since during phase 2 flow often continues to increase after the intestine has reached its maximum enlargement (see fig. 4), and since full compensation may be maintained throughout phase 2 in the face of a delayed tone rise in the intestine which reduces its volume (see fig. 1).

The second alternative seems the more probable. The mechanism set in operation by stretch might conceivably be entirely extravascular, if the following conditions, most of which are improbable, existed: *1*, the total blood flow through the system intestine-mesentery were reciprocally related to the tonus of the outer layers of muscle in the intestinal wall; *2*, the tonus of these outer layers were inhibited by stretch; *3*, the inner layers of the intestinal wall resisted stretch to the extent that during distention (or stretching) extravascular pressure in the outer layers, under the operation of mechanism *2*, actually fell below normal. A vascular mechanism fits the data equally well, and involves less unlikely assumptions. It leaves unexplained only the apparent relationship of the compensating mechanism to intestinal muscle tone, which is being studied further.

It is suggested as a working hypothesis that the stretching of the intestinal walls during distention sets in operation, through the peripheral nervous apparatus, a mechanism which compensates for the rise in extravascular pressure. The ultimate compensating mechanism is probably dilatation of arterioles. Assuming that no redistribution of flow occurs in the distended intestine, such a mechanism would permit a rise in peripheral intravascular pressure so as to prevent compression of the smallest vessels.

On the basis of this theory, the critical level of distention for which compensation could be effected would be determined by the maximum elevation of capillary pressure permitted by full arteriolar dilatation, as well as by the irritability and efficiency of the compensating mechanism itself.

SUMMARY AND CONCLUSIONS

Blood flow through loops of the small intestine of dogs anesthetized with barbital is only momentarily interrupted by inflation of the loop under pressures below 30 mm. Hg. Higher inflation pressures below mean mesenteric arterial pressure cause an initial marked reduction in flow, from which there is partial recovery within a few seconds.

Simple transverse stretching of a strip of intestine opened along its antimesenteric border sometimes causes a simple increase in flow through the strip.

If enlargement of the loop is prevented by encasing the loop in plaster-of-Paris, or if the loop is treated with cocaine or procaine, any rise in lumen pressure causes reduction in flow which persists without any tendency toward recovery, or with reduced recovery, throughout the inflation.

On deflation of the untreated loop a period of hyperemia ensues, sometimes interrupted early by a period of reduced flow apparently due to deflation hypermotility. The increased flow following deflation appears not to be identical with reactive hyperemia in its mechanism, since it is abolished by treating the gut with cocaine or encasing it in plaster, procedures which augment the flow deficit incurred during inflation, and which do not prevent reactive hyperemia following arterial occlusion.

No phase of the response to inflation is significantly modified by perivascular mesenteric denervation.

It is suggested as a working hypothesis that stretching the walls of the intestine during inflation sets up vasodilatation through intrinsic nervous mechanisms, resulting in local circulatory compensation for the added resistance to flow.

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EFFECTS OF INJECTION OF EXTRACT OF YEAST ON GASTRIC SECRETION IN DOGS[1,2]

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We have prepared a concentrated aqueous acid extract of yeast which, when injected intramuscularly, increases the volume and acidity of secretion by previously resting stomachs of dogs. A similar preparation from spinach has been reported by Bickel (1917), and Gleichmann (1934) observed gastric stimulation in dogs when filtered tomato juice was injected intramuscularly. In no case has the gastrin-like principle been isolated, although the active material from spinach has been characterized as stable to dry heat up to about 140° and to boiling with concentrated hydrochloric acid.

Methods and general procedure. *Preparation of yeast extract.* Commercial foil-wrapped cake yeast was extracted exactly according to the procedure of Keeton and Koch (1915) for extraction of "gastrin" from animal tissues. The final dry residue was taken up in water so that 1 cc. of solution represented 10 grams of original yeast.

Animals.[3] Our data represent 6 dogs, weighing from 8 to 13 kilos. Three dogs were prepared with a gastric fistula of the Spivack type, while in 3 dogs the gastric fistula was made with an isolated section of jejunum. These 6 dogs gave negative tests for free hydrochloric acid and very low pepsin values in more than 50 per cent of control gastric samples. In 4 additional dogs consistently positive effects upon gastric secretion were obtained by injection of histamine or the yeast extract. However, since the latter dogs regularly secreted an acid gastric juice in control periods, data of these experiments are not included.

Experimental. In all experiments the dogs had received no food for 16 hours. Gastric contents were collected by a syringe and a no. 20 rubber catheter inserted through the fistula. At the beginning of an experiment, and at 15 minute intervals during the succeeding 2 hours, the

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stomach was thus emptied. The 2 samples collected during each 30 minute interval were combined for analysis. At the end of the first 30 minute interval, which served as control, the injection of test material was given intramuscularly.

Analytical methods. For *free hydrochloric acid*, 2.5 to 5.0 cc. of the gastric sample were titrated to pH 2.4 (thymol blue indicator); in the same sample *total acid* was measured by further titration to the second color change of thymol blue at pH 8 (Harrison, 1930). *Pepsin* was estimated in the gastric samples when sufficient additional material was available. The method for pepsin was adapted from the amino titration procedure of Linderstrøm-Lang (1928) and the method of Andersen (1938). One cubic centimeter of the gastric sample was incubated at 38° for 100 minutes with 5.0 cc. of 5 per cent sodium caseinate and 1.00 cc. of 0.75 N hydrochloric acid. A control incubation was made with 1.00 cc. of the gastric sample which had been heated in a boiling water bath for 2 minutes. Peptic activity was related to the difference in amino titration values of the two systems. One pepsin unit has been defined arbitrarily as that amount which effects the liberation of one-tenth milliequivalent of amino acid under the specified condition.

RESULTS. Averages of data from 12 experiments in which 3 or 4 cc. of yeast extract[4] were injected intramuscularly are shown in table 1. In the same table are included for comparison the average data of 16 control experiments on the same dogs in which sterile 0.9 per cent sodium chloride solution instead of yeast extract was injected, and the average results of 16 experiments in which 0.1 mgm. of histamine was given intramuscularly. Probable errors of the means are recorded in table 1 for all the volume, free hydrochloric acid, and total acid data and for the pepsin data of the first 30 minute period after injection. The remaining pepsin data are from so few observations as to be useless for statistical analysis.

As compared with gastric response to control injections of 0.9 per cent sodium chloride solution, significant increases occur in volume, free hydrochloric acid, and total acid of the samples collected during the first 30 minute period after injection of yeast extract or histamine. Any differences between the effects of yeast extract and of histamine during this period are of doubtful significance on the basis of this series of data. Free hydrochloric acid and total acid secretion during the second 30 minute period after histamine is still significantly above control levels, but increase in average volume for this period is probable only in the yeast extract experiments. All other apparent variations from the controls in acidity or volume, in any period, are not statistically significant.

[4] Orientation experiments had shown that smaller doses of yeast extract frequently failed to produce a secretory response.

In our judgment, pepsin changes are of doubtful meaning in all experiments, although *average* pepsin values during the first 30 minute period after injection of yeast extract represent a probable increase as compared with controls. Such dogs are capable of secreting 10 to 20 units of pepsin within each of three consecutive 30 minute periods following a more specific stimulus which does not evoke a correspondingly larger secretion of acid. The values for pepsin noted in table 1, small as they are, may be attributed in part at least to mechanical stimulation by the catheter, since some output of pepsin is found after injection of 0.9 per cent sodium chloride.

TABLE 1

Effects of injection of 0.9 per cent sodium chloride, histamine, and yeast extract on gastric secretion

INJECTION	VOLUME	FREE HCl	TOTAL ACID	PEPSIN
30 minute period preceding injection				
	cc.	*meq.*	*meq.*	*units*
0.9 per cent NaCl	7.3 ± 1.4	0.01 ± 0.00	0.27 ± 0.04	0
0.1 mgm. histamine	10.1 ± 1.9	0.03 ± 0.01	0.47 ± 0.10	0
3 or 4 cc. yeast extract	5.4 ± 0.8	0.06 ± 0.03	0.61 ± 0.23	0.9
First 30 minute period after injection				
0.9 per cent NaCl	7.1 ± 1.6	0.02 ± 0.01	0.41 ± 0.11	0.18 ± 0.11
0.1 mgm. histamine	21.8 ± 2.1	0.89 ± 0.13	2.04 ± 0.18	0.67 ± 0.12
3 or 4 cc. yeast extract	32.3 ± 2.9	1.09 ± 0.20	2.09 ± 0.26	2.3 ± 0.5
Second 30 minute period after injection				
0.9 per cent NaCl	5.5 ± 0.9	0.01 ± 0.01	0.29 ± 0.12	0.8
0.1 mgm. histamine	12.0 ± 1.8	0.17 ± 0.05	0.84 ± 0.09	0.9
3 or 4 cc. yeast extract	17.5 ± 3.0	0.61 ± 0.21	1.38 ± 0.31	1.4
Third 30 minute period after injection				
0.9 per cent NaCl	5.0 ± 0.7	0.04 ± 0.02	0.70 ± 0.13	1.4
0.1 mgm. histamine	6.3 ± 1.0	0.05 ± 0.03	0.43 ± 0.24	1.1
3 or 4 cc. yeast extract	10.0 ± 2.0	0.18 ± 0.09	1.00 ± 0.35	1.7

In 6 experiments with these same dogs the injection of 4.5 mgm. of thiamine chloride failed to elicit any more gastric response than followed the injection of 0.9 per cent sodium chloride.

In an effort to determine whether the acid-stimulating factor in our yeast extracts was histamine, both blood pressure effects and chemical behavior have been tested, as follows, but with inconclusive results:

Into an ether anesthetized cat was injected intravenously 0.1 cc. of yeast extract diluted to 1.0 cc. with 0.9 per cent sodium chloride. The resulting

decrease in blood pressure was approximately the same as followed the injection of 0.005 mgm. of histamine in 1.0 cc. of 0.9 per cent sodium chloride. However, after 12 mgm. of atropine, effect of yeast extract had been practically abolished, whereas the histamine depressor effect was still considerable.[5]

When treated with the Pauly reagent by the method of Jorpes (1932) 1.0 cc. of the yeast extract develops a color whose absorption at 495 mμ in the spectrophotometer is equivalent to that of 0.03 mgm. of histamine under like conditions. Whereas the color developed by histamine absorbs maximally at 495 mμ, the yeast extract coloration absorbs much more strongly at 485 mμ.

It may be noted that Lautenschläger (1938) in a review of the industrial significance of yeast has reported the laboratory preparation of histamine from the proteins of yeast and from the products of their decomposition.

SUMMARY

A concentrated extract of fresh yeast has been prepared which, when injected intramuscularly, stimulates gastric secretion in a histamine-like manner. That quantity of extract representing 30 to 40 grams of yeast has an activity equivalent to 0.1 mgm. of histamine. Injection of 4.5 mgm. of thiamine chloride was without effect. While such tests as have been made suggest that the active principle of the yeast extract is not histamine, the evidence on this point must be considered as inconclusive.

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[5] We are indebted to Prof. O. S. Gibbs, of the Department of Pharmacology, for assistance in this assay.

THE EFFECT OF HIGH PRESSURE TREATMENT ON THE PHYSIOLOGICAL ACTIVITY OF INSULIN

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Many of the chemical and physical properties that affect the physiological activity of insulin have been summarized by Jensen (1). The writers are not aware, however, of any study of the effect of hydrostatic pressure on the physiological activity. Several researches have shown that high pressure is an effective means of controlling many reactions of biological and bio-chemical interest. Bridgman (2) in 1914 reported that egg albumin is coagulated by high pressure and with Conant (3) in 1929 found that carboxyhemoglobin is denatured under similar conditions. More recently, Bassett and his collaborators (4) have reported the effects of high pressure on several bacteria, vaccines, and enzymes but the data are of a preliminary nature and few conclusions can be made. Investigations in the high pressure laboratory of the Department of Physics of this College by Dow and Matthews (5) and by Matthews, Dow and Anderson (6) have demonstrated that denaturation of proteins by high pressure results in their deactivation. The extent of denaturation depends on the magnitude of the pressure and the time of exposure, as well as on several chemical factors, for example, the pH of the solution.

Since it is generally agreed that insulin is a typical protein, it would be of interest to study its denaturation at high pressure in relation to its physiological activity.

Experimental method. Insulin solutions from two sources were used in these experiments. Lilly's U-20 Iletin was used in the major part of the investigations and some study was made with amorphous insulin (17 units per mgm.) from the Banting Institute.

The apparatus for hydrostatic pressure treatment is similar to that used extensively by Bridgman (7) and by the senior author in investigating various physical and chemical behavior at high pressure. Initial pressures produced by a hand-pump are further intensified by a hydraulic press that generates high pressure in the test chamber. Pressure can be increased or reduced at such a slow rate that there is no sensible change of temperature during the process. A thermostated bath can be placed about the test chamber to keep it at constant temperature, although in these experi-

ments it was not considered essential and the treatments were applied at room temperature.

The sample of insulin was placed in one of two types of containers. Flexible tooth paste tubes lined with paraffin were first tried as containers. The tubes were used by soldering the lower ends, filling with the test sample, and then sealing by means of the threaded cap. Consequently the sample was separated mechanically from the kerosene that transmitted pressure in the test chamber. A brass tube, lined similarly with paraffin and closed at one end, served as the other type of container. As the samples did not completely fill the tube, paraffin oil was poured in to cover the insulin with a layer of about one inch in depth. Other experiments had shown previously that good separation of liquids was obtained in this way and that similar results were found irrespective of the nature of the container. This is similar to the experience of Basset (4). Both containers were used indiscriminately in these experiments but most of the tests were made with the latter type.

The physiological activity of both normal and pressure treated insulin was measured by the percentage lowering of blood sugar in rabbits that were given standard doses, and doses of the pressure treated samples that were considered the equivalent of standard doses. A standard solution was considered to have 2.5 physiological units per cubic centimeter of solution and was made up with U-20 Iletin in physiological salt solution. A standard dose was taken as 1 cc. of solution that contained 2.5 units per 2 kgm. of body weight. The pressure treated samples were made up in the same way by using the same quantity of treated insulin in physiological salt solution. For the pressure treatment, however, the Iletin was used as it was received from the maker but the amorphous insulin was made up in physiological salt solution to contain 140.1 units per cubic centimeter. While the onset of convulsions, as well as the intensity and length of them, were observed they were not considered sufficiently reliable measure of the insulin activity to be recorded in this paper. One reason for this was the wide variation of the convulsive level in the rabbits.

The animals were not ideal for the purposes of the experiments. They were of mixed breed and evidently varied considerably in physical stamina. Several of their ears were unsuitable for bleeding. Two females and ten males were used in the experimental procedure. Of the former, rabbit 7 unexpectedly gave birth prematurely to a litter during one of the tests. She had been used previously several times, giving consistent behavior as can be judged by the data. Culhane (8) has reported that females having a litter are generally unsuited for tests. It is thus surprising to observe such consistent results with a pregnant rabbit. The ears of the other female were so unsuited for bleeding that she could not be used after a preliminary experiment.

The method of "cross-test" advocated by Culhane (8) was used in the

experiments. By this procedure one group of animals is given the standard preparation and another group the test sample. This method was followed twice a week using four animals for each test but each animal was not used more than once a week in most cases. Two rabbits were usually given the standard insulin dose and two others the pressure treated sample. The tests were carried out over a period of five weeks.

The diet prescribed for the rabbits consisted of hay and oats. The rabbits were without food 24 hours prior to the experiments. During this period, as well as during the tests, they were allowed an ample amount

TABLE 1

Percentage sugar decrease in rabbits inoculated with pressure treated and untreated iletin

ANIMAL	PER CENT CHANGE UNTREATED ILETIN	PER CENT CHANGE WITH PRESSURE TREATED ILETIN					AVERAGE PER CENT CHANGE OF PRESSURE TREATED ILETIN	PER CENT DIFFERENCE TREATED-UNTREATED ILETIN
		1 5,000 kgm./cm².	2 5,000 kgm./cm².	3 10,000 kgm./cm².	4 10,000 kgm./cm².	5 10,000 kgm./cm².		
1	45		30	17	46	43	32	−13
2	No results							
3	71	71	73 Died				72	+1
4	47		31	36			34	−13
5	55*	65	74	51	Died		63	+8
6	56		51	43	39		44	−12
7	55*		59	45	47		50	−5
8	57*			38 53**	64	57	53	−4
9	60	64	66	40	69	59	60	0
10	58	70	77 Died				74	16
11	63				46	62	54	−9
12	No results							
Average								−5

* Two determinations differing by 1%.
** Amorphous insulin treated for 3 hrs.

of water. After inoculation samples of blood were drawn from the ears at $1\frac{1}{2}$, 3 and 5 hours, respectively. These together with the blood sample that was always taken before inoculation were sufficient to allow the change in blood sugar to be followed with some accuracy. The micromethod of Folin and Malmros (9) that requires only 0.1 cc. of blood was used to determine the blood sugar content.

Data and results. The experimental results summarized in table 1 were obtained with Iletin, except in one case as indicated. The maximum percentage decrease in blood sugar level is recorded for normal insulin and

pressure treated samples at 5,000 and 10,000 kgm/cm^2. Two insulin samples were treated at 5,000 kgm/cm^2. The exposures, tests 1 and 2, were for 3 and 15 hours, respectively. Three treatments at 10,000 kgm/cm^2, tests 3, 4 and 5, were made for 15, 23 and 41 hours, respectively. To determine the maximum percentage decrease in sugar level, the four sugar determinations, measured as the number of milligrams per 100 cc. of blood, were plotted graphically and the maximum decrease was read from the resulting curve in each case. This method was adopted since it was found that the curves were always similar but not of a simple nature. Since they showed a definite relation in respect to time, a simple average of sugar determinations during the 5 hour period would have little significance.

While it can be shown that the average of the percentage decreases for the treated samples is about 5 per cent less than the average for untreated insulin, this difference does not prove necessarily that the physiological activity of insulin is reduced by pressure treatment. Considering that the animals used in this study were not the best for the purpose, and the fact that relatively few were available for the tests, it would appear reasonable to assume that 5 per cent represents a likely error in these experiments. On this basis the writers are inclined to state that pressure treatment had no effect on the physiological activity of insulin.

Although the pressure treatments did not change the appearance of Iletin, it was realized that with stronger concentrations of insulin, and particularly with insulin that contained no preservative, pressure might produce coagulation as had been observed previously (6) in the case of pepsin. To verify this the amorphous insulin in physiological salt solution was subjected to a pressure treatment of 10,000 kgm/cm^2 for 3 hours. In this case, however, the treatment did produce coagulation that was clearly visible. The precipitate appeared similar to the appearance of egg albumin, or any other typical protein, such as pepsin, when it is coagulated by heat. The precipitate was carefully mixed with additional salt solution to a dilution corresponding to the previous doses and then injected into a starved rabbit. The animal was no. 8 that had been used in the previous experiments. The maximum percentage decrease in sugar was found to be 53 per cent, a value close to that found for the animal previously (table 1). The physiological activity of amorphous insulin was clearly not affected by the fact that coagulation resulted from the pressure treatment.

Van Slyke amino nitrogen determinations were made on both the control solution of amorphous insulin and the pressure treated sample with the result that no change in amino nitrogen was detected. Consequently there is no evidence of hydrolysis by pressure.

Discussion. It is well known that insulin loses its physiological

potency when denatured by heat. In this respect it behaves similarly to many other proteins. The present experiments show, however, that while insulin may be denatured by pressure, as indicated by coagulation in concentrated solutions, the treatment does not reduce its physiological activity. Evidently denaturation caused by pressure must be fundamentally different from that brought about by heat. Since when applying pressure to a sample the rate of compression can be so low that there is no appreciable rise of temperature, the process can be said to be carried out isothermally. It is important to note that during such a compression more energy flows out of the sample in the form of heat than is put in by the work of compression. This is easily understood when it is recalled that the attractive forces in the liquid must do work during the compression. It has been shown previously (6) that by compressing water isothermally to a pressure of 1,000 kgm/cm.^{2}, about seven times more energy flows out of the water than is put in by the compression. When it is remembered that in denaturing by heat energy must always flow into the sample, as indicated by the temperature rise, it is evident that in pressure denaturation the physical process insofar as energy is concerned, is decidedly different. This appears to be a significant factor that has not been studied thoroughly. It suggests many possibilities for future study since it may involve some reaction within the protein molecule that is not known at present.

The recent theory of Wrinch (10, 11, 12) on the structure of proteins has given impetus to speculations on the nature of the changes that take place in denaturation and hydrolysis. According to this theory denaturation takes place when the cyclol bonds are broken, but the polypeptide chains remain uneffected. A breaking of a peptide link, however, involves a reaction with another molecule and is to be associated with hydrolysis. According to this interpretation, the pressure denaturation observed in these experiments broke the cyclol bonds in the insulin molecules with the result that the polypeptide chains became the predominating type of structure. No change in amino nitrogen would be expected unless hydrolysis occurred. Since no change of activity has been observed in these experiments, it would appear that the physiological properties of insulin are bound up in some manner with the polypeptide linkage in the molecule, denaturated or otherwise. The possibility of associating the specific physiological properties of insulin with its component amino acids has been discussed by Jensen (13).

The authors wish to acknowledge the donation of the Iletin by Dr. W. B. Peck of the Lilly Research Laboratories and the amorphous insulin by Professor W. R. Franks, of the Banting Institute. They are indebted to Dr. A. K. Anderson of this college for his kindness in putting his equipment at their disposal.

SUMMARY

The physiological activity of insulin is unaffected by long exposure to pressure of the magnitude of 10,000 kgm/cm^2. Activity was measured by observing the percentage decrease of blood sugar level in rabbits.

Evidence that pressure produced denaturation is given by the coagulation of insulin. Coagulation does not affect the physiological activity. The absence of any change in amino nitrogen content of the insulin has been taken to mean that pressure does not produce hydrolysis under these conditions.

It is suggested that the results of these experiments indicate that the physiological activity of insulin is associated with its polypeptide or amino acid linkages, rather than with any particular bonding or grouping of the chains such as might exist in the undenaturated state of the molecule.

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DEHYDROGENASE INACTIVATION IN OXYGEN POISONING[1]

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Libbrecht and Massart (1937), using the Warburg technique, demonstrated a decreased oxygen uptake of a succino-dehydrogenase system exposed to oxygen at high pressures. They concluded that the high concentrations of oxygen inactivated the dehydrogenase system. We have been interested in the possible rôle this dehydrogenase inactivation might play in the induction of the toxic effects of oxygen at increased barometric pressure on various tissues. Such toxic effects are exemplified by a decrease in the strength of contraction of striated muscle (Bean and Bohr, 1938), an upset in the pace setting mechanism of the isolated heart (Bohr and Bean, 1939), and a fall in the tonus and loss of rhythmicity of isolated non-striated muscle (Bean and Bohr, 1940). In each of these tissues the return to oxygen at atmospheric pressure is attended by a complete or partial recovery of normal function, the degree of recovery being conditioned by the severity and duration of the exposure to increased pressure. Where the tissue is exposed a second time these deleterious effects have a more rapid onset, are more pronounced and recovery—if any—invariably incomplete.

In our attempt to determine whether the influence of oxygen at high barometric pressure on isolated tissue might possibly be related to an inactivation of dehydrogenase, the Thunberg methylene blue reaction (Thunberg, 1920) was used as an index of dehydrogenase extract activity. The changes of such activity induced by exposure of the extract to oxygen at increased barometric pressure of various intensities and durations, were followed, as well as the possible recovery of the activity after the return of the extract to oxygen at atmospheric pressure.

In our experiments a dehydrogenase-cytochrene system was prepared following the method of Stotz and Hastings (1937), but pork hearts, weighing close to 200 grams, were used instead of beef hearts. The extraction was carried out on the day of the killing, and the extract was preserved by overlaying with toluene and storage in an icebox at 5°C. Although there was no significant decrease in activity of the enzyme solution preserved

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in this manner after a week's storage, all of the experiments referred to in this report were carried out within 48 hours after the preparation of the extract. This precaution was taken in view of the rapidly deleterious effect of ageing on the oxygen activating systems, and the important part that this system is said to play in the inactivation of dehydrogenase by high oxygen (Libbrecht and Massart, 1937).

A tonometer rotated by a small electric motor mounted inside of the compression chamber was used to equilibrate the extract with the desired pressure of oxygen. Rapid equilibration was assured by using only a small volume of the extract (10 cc.) in a large tonometer (200 cc.). The temperature of the chamber was maintained at 37°C. and the oxygen used in compression was saturated with water to prevent concentration of the extract. Similar methods were used to equilibrate the extract with the pure nitrogen or air used as control gases.

In preliminary experiments we found that vigorous mechanical agitation as produced by bubbling the extract solution with gas caused a decrease in dehydrogenase activity. That such a decrease in activity during bubbling was due to mechanical agitation rather than to any peculiarity of the gas employed, was shown by the fact that it occurred as a result of bubbling, not only with oxygen, but also with air and pure nitrogen. The degree of the inactivation of the dehydrogenase so induced is apparently dependent upon the violence and duration of the agitation, for it was found that if equilibration of the extract with air or pure nitrogen was accomplished by very gentle rotation of the tonometer, no diminution in the activity of dehydrogenase occurred. In light of these findings precautions to eliminate so far as possible any mechanical agitation of the extract assume great importance. In all of our experiments, therefore, equilibration of the extract was accomplished by gentle rotation of the tonometer, not only during exposure to increased pressure, but also during decompression to facilitate the escape of dissolved gas without the formation of bubbles in the fluid.

Immediately after a given exposure to oxygen at increased barometric pressure or to the control gas, and before the methylene blue reaction mixture was made up, the extract was placed in a 250 cc. flask and evacuated just to the boiling point at 37°C. This negative pressure was maintained for a period not longer than 1 minute, during which time the flask was gently swirled to promote rapid liberation of oxygen from the solution. In this manner the oxygen tensions of the test and control extracts were adjusted to the same level. Such deoxygenation is essential since oxygen, if left in solution, will itself act as a hydrogen acceptor and thus cause an increase in the reduction time of methylene blue. To eliminate the possible involvement of the influence of mechanical agitation on the dehydrogenase activity in this procedure, the precautions against violent

and prolonged agitation as mentioned above were rigidly observed. It was demonstrated that this method of deoxygenation did not alter the dehydrogenase activity.

The reaction mixture used in testing the activity of the dehydrogenase factor after a given exposure to the gas was essentially similar to the standard reaction mixture as used by Lehman (1930). The dehydrogenase extract was diluted (with a $\frac{1}{15}$ M. NaH_2PO_4-Na_2HPO_4 buffer, pH 7.29) in order to give a methylene blue decolorization time between 10 and 15 minutes. The Thunberg tubes, containing the reaction mixtures indicated in the tables below, were placed in a 37°C. water bath and evacuated with a "Cenco-Hyvac" vacuum pump. The time for decolorization of methylene blue was taken from the moment boiling commenced.

TABLE 1

Heart A

TUBE NO.	TUBE CONTENT				METHYLENE BLUE REDUCTION TIME
	0.0004 M. meth. blue	0.2 M. succinate	Control extract (air at atm. pressure)	Test extract (O_2 at 7.6 atms. pressure)	
	cc.	*cc.*	*cc.*	*cc.*	*min.*
1	0.3	0.2	2.5		12
2	0.3	0.2		2.5	19.5
3	0.3	0.2	2.5		12
4	0.3	0.2		2.5	20.5
5	0.3	0.2	2.5		12
6	0.3	0.2		2.5	19.5

The first table demonstrates a typical alteration in activity of the succino-dehydrogenase system produced by exposure to oxygen at 100 pounds' pressure (7.6 atmospheres).

In this series, exposure of the test extract (tubes 2, 4, and 6) to oxygen at 100 pounds' pressure (7.6 atms.) for one and one-half hours caused a 40 per cent inactivation of the dehydrogenase as measured in methylene blue reduction time.

It is noteworthy that the degree of inactivation produced by high oxygen varied from one preparation to the next. For example, in extracts of four different hearts inactivations of 50 per cent, 42 per cent, 9 per cent and 20 per cent were produced by two and one-half hours' exposures to oxygen at 100 pounds' pressure. Yet repeated exposures of extracts from the same heart gave consistent degrees of inactivation. This variation between extracts from different hearts is of interest in view of the striking differences in susceptibility of intact animals to oxygen poisoning. Such differences have been attributed by some investigators to the age (Massart, 1936), the state of nutrition (de Almeida, 1934), and the thyroid gland activity

(Campbell, 1938) of the animal. Youth, starvation and thyroidectomy are said to be of protective value against oxygen poisoning. The inconstancy in the susceptibility of the dehydrogenase extracts from different animals to inactivation by oxygen at high barometric pressure as shown in our experiments may represent an additional basis for the differences in the susceptibility of the intact animal to oxygen poisoning. On the other hand, it is conceivable that the differences explained as due to age, nutrition and thyroid activity are in reality due to corresponding conditions of the dehydrogenase system or its environment which alter its susceptibility to oxygen at high pressure. For example, the dehydrogenase system which governs a particular reaction in the metabolism of the growing cell (i.e., in youth) might be expected to differ from that system in the mature cell and it may be that the enzyme during the metabolism of growth is

TABLE 2

Heart A

TUBE NO.	TUBE CONTENT					METHYLENE BLUE REDUCTION TIME
	0.0004 M. meth. blue	0.2 M. succinate	Control (fresh from icebox)	O_2 atm. pressure	7.6 atms. pressure (N_2)	
	cc.	*cc.*	*cc.*		*cc.*	*min.*
1	0.3	0.2		2.5		13
2	0.3	0.2	2.5			13
3	0.3	0.2			2.5	13.5
4	0.3	0.2		2.5		13
5	0.3	0.2	2.5			13
6	0.3	0.2			2.5	13.5
7	0.3	0.2		2.5		13
8	0.3	0.2	2.5			13

more resistant to inactivation by high oxygen pressure than the enzyme of the mature cell. A study of the resistance of dehydrogenase systems from animals of different age groups would be of interest in this connection.

With the purpose of determining the effect of oxygen at atmospheric pressure and of 100 pounds' pressure *per se* on the activities of the dehydrogenase extract the following experiment was carried out.

The extract used in this experiment was taken from the same heart as was that used in the first experiment—the results of which are shown in table 1. The "control" extract of the above résumé (table 2) was kept in the icebox, while the other two were equilibrated in tonometers for one and one-half hours—one with oxygen at atmospheric pressure, and the other with nitrogen at 100 pounds' pressure (7.6 atms.). These control measures did not cause an appreciable alteration in dehydrogenase activity.

As noted above, isolated tissues suffering from the deleterious effects of high oxygen pressure frequently regained their normal function when the

oxygen concentration was reduced to atmospheric pressure. Such recovery was usually maximal within one-half hour after decompression to atmospheric pressure. In order to determine whether this recovery of normal tissue activity might not be explained on the basis of a concomitant recovery of dehydrogenase activity, the following experiment was performed: After the activity of the extract had been diminished by exposure to oxygen at 100 pounds' pressure (7.6 atms.) for one hour, the pressure was reduced to atmospheric; the extract evacuated and gently swirled and its activity determined at fifteen minutes and at one hour following the decompression. The results of such determinations are summarized in table 3 below.

TABLE 3

Heart B

TUBE NO.	TUBE CONTENT				METHYLENE BLUE REDUCTION TIME
	0.0004 M. meth. blue	0.2 M. succinate	Air control extract atm. pressure	Extract exposed to O_2 at 7.6 atms.	
	15 minutes after decompression				
	cc.	*cc.*			*min.*
1	0.3	0.2	2.5		7
2	0.3	0.2		2.5	9.5
3	0.3	0.2	2.5		7.5
4	0.3	0.2		2.5	9
5	0.3	0.2	2.5		7.5
	One hour after decompression				
1	0.3	0.2	2.5		7
2	0.3	0.2		2.5	9.8
3	0.3	0.2	2.5		7
4	0.3	0.2		2.5	9
5	0.3	0.2	2.5		7

Even one hour after decompression from an exposure to oxygen at 100 pounds' pressure the test extract (tubes 2 and 4) showed no signs of spontaneous recovery. Several explanations might be offered for this lack of parallelism between the apparent recovery seen in isolated tissues following decompression from high oxygen and the absence of recovery in the dehydrogenase system *in vitro*. It may be that either some element capable of reactivating the dehydrogenase system present in isolated tissue is missing in the extract preparations, or that the reversible decrease of normal function of the isolated tissue exposed to high oxygen is based on some reversible process which does not involve the dehydrogenase system. A third possibility presents itself when we consider the fact that

although the isolated tissues seem to have regained normal functional activity after an initial exposure to high oxygen, a second exposure causes a more rapid onset of the deleterious effects, the recovery from which is usually less than complete. Thus, in spite of the apparent return to normal function after the first decompression, there must be some mechanism that has not shared such recovery. It is here that the irreversible inactivation of this dehydrogenase system may fit into the picture of oxygen poisoning.

SUMMARY

Experiments are described in which the effects of oxygen at high barometric pressure (7.6 atms.) on succino-dehydrogenase extracts of pork hearts were determined.

The importance of elimination of violent or prolonged mechanical agitation of the extract, such as that of bubbling during its equilibration with the overlying gas, is stressed since it was found that such agitation in itself may alter dehydrogenase activity.

Exposure of the extracts from different hearts to oxygen at 100 pounds' pressure (7.6 atms.) for two and one-half hours resulted in decreased activity of the enzyme of from 9 per cent to 50 per cent. It is suggested that the variability in degree of inactivation might be one underlying cause for the lack of uniformity in susceptibility of intact animals to oxygen poisoning.

The inactivation of the dehydrogenase system in these *in vitro* experiments appears to be an irreversible process. The relationship between this apparent irreversible inactivation of dehydrogenase extract and the degree of functional recovery which occurs in isolated tissue preparations following exposure to high pressures of oxygen is discussed.

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DIFFERENTIAL SENSITIZATION OF ADRENERGIC NEURO-EFFECTOR SYSTEMS BY THYROID HORMONE[1]

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Sensitization of the sympathetic nervous system to epinephrine is a common explanation for various signs and symptoms encountered in hyperthyroid states (19). Among the symptoms of hyperthyroidism which would be explained at least in part if adrenergically innervated effectors were sensitized to adrenalin by thyroid hormone are functional exophthalmos, widened lid-slits, von Graefe's sign, dilated pupil, tachycardia, the Goetsch epinephrine test, and possibly some of the disturbances in carbohydrate metabolism. On the other hand, certain fairly common symptoms of thyrotoxicosis are the reverse of what would be expected if all adrenergically innervated effectors were sensitized. Included in these latter symptoms are diarrhea, excessive hunger, and dilatation of skin vessels. Means (10) has indicated that virtually all of the symptoms of hyperthyroidism could be produced by hypermetabolism *per se*. This being the case, postulation of sensitization of the sympathetic nervous system would be unnecessary.

The sympathetic system includes at least four physiological types of neuro-effector systems, namely: excitatory adrenergic (EA), inhibitory adrenergic (IA), excitatory cholinergic (EC), and inhibitory cholinergic (IC). A review of the extensive literature on the thyroid-autonomic relationship reveals that, with few exceptions, studies of sensitization of sympathetically innervated effectors to chemical mediators by thyroid hormone have been concerned with sensitization to adrenalin in the EA type of system. The most frequently used indicators have been the blood pressure, the caliber of skin vessels, and the heart rate. Although opposite results have been obtained, the majority of these studies indicate that in thyrotoxic animals certain effectors receiving an excitatory adrenergic innervation show a hypersensitivity to adrenalin. Some of the literature on the effect of thyroid hormone on the heart rate will be cited since the S-A node was used as an indicator in this study.

McIntyre (9) has reported an essentially normal degree of acceleration of the denervated dog heart as a result of thyroid feeding. A direct

[1] Aided by a grant from the John and Mary R. Markle Foundation.

accelerator effect of thyroxin is also indicated by persistence of the fast rate in the denervated (9) or in the isolated (6) thyrotoxic heart. In the latter experiments epinephrine was no more effective in the thyroxinized, isolated hearts than in the normal. A greater than normal accelerator response to adrenalin in chronically thyrotoxic hearts has been reported for cats (14), frogs (4), and terrapins (8). In electrocardiographic studies Rosenblum et al. (13) found the thyrotoxic rabbit heart more susceptible than normal hearts to the irregularity-producing effects of adrenalin. Administration of thyroid substance to man exaggerates the cardiovascular reaction to adrenalin (7). The above studies show that a considerable part of the tachycardia observed in an intact thyrotoxic animal is explicable on the bases of increased demands on the heart accompanying the rise in total metabolism and a direct stimulatory action of thyroid hormone on the heart. Heightened sensitivity to epinephrine and to the adrenergic mediator appears to be a third factor which would tend to increase the lability of the accelerator mechanism rather than exert much influence on the basal rate.

Apparently no study has been made to determine if *inhibitory* adrenergic neuro-effector systems in intact animals are sensitized by thyroid hormone. However, various facts are suggestive. The smooth muscle of the non-sphincteric parts of the stomach and intestine receives an inhibitory adrenergic innervation from the sympathetic nervous system (20), (23), (24). Clinical observations indicate that the motility of the gastro-intestinal tract tends to be reduced in hypothyroidism (1), (18) and increased in hyperthyroidism (15), (16). Fetter and Carlson (3) found that hyperthyroidism stimulated gastro-intestinal motility in experimental animals, and they cited previous literature supporting this view. Recently Morrison and Feldman (11) have reported that induced hyperthyroidism increases gastro-intestinal motility with or without sectioning of the vagus nerve. Such facts argue against but do not disprove sensitization of tonically active inhibitory adrenergic intestinal nerves by thyroxin.

Since adrenalin is considered to be the mediator produced at adrenergic nerve endings, this substance should be more inhibitory to the intestine of thyrotoxic animals than of normal animals if thyroid hormone sensitizes the entire sympathetic nervous system. Experiments on the isolated rabbit intestine have failed to show any sensitization to the inhibitory effects of adrenalin by thyroid hormone (5) (12). The experiments described below show that the dog intestine "in situ" undergoes no increased sensitivity to adrenalin during a period of thyroid feeding which produces the characteristic cardiac sensitization.

Methods. Records of the following were obtained from each of five dogs before commencement of thyroid feeding. 1. The heart rate under near-basal conditions. 2. Minimal cardio-accelerator dose of adrenalin,

and the effects on heart rate of doses two to four times as great. 3. Motility of a Thiry fistula of the jejunum. 4. Minimal intestine-inhibiting injection rate of adrenalin, and inhibitory effect of doses two to four times as great. 5. Degree of reflex inhibition of the intestine by adrenergic nerves during rectal stimulation. 6. The intestinal responses to the sympathomimetic substances reflexly liberated during acetylcholine hypotension. Records of heart rate were taken with an electrocardiograph, and records of intestinal motility were taken by a balloon-mercury-manometer system. After completion of the above analysis of the sensitivity of the sino-auricular node (EA innervation) and of intestinal smooth muscle (IA innervation) to injected and reflexly liberated sympathomimetic substances, the animals were fed 0.8 to 1.2 grams of powdered thyroid (Armour & Co.) per kilo daily for a period of two to five weeks. Procedures 1 to 6 were then duplicated in the thyrotoxic animals to evaluate alterations in sensitivity of the two types of adrenergic neuro-effector systems.

RESULTS. 1. *Effect of thyroid hormone on the sensitivity of the cardio-accelerator mechanism (EA) to adrenalin.* Four of the five thyroid-fed dogs developed the general signs indicative of thyrotoxicosis. These consisted of warm skin, high basal heart rates, hyper-excitability, excessive cardiac acceleration and panting with mild exercise. One of the five dogs developed diarrhea and failed to show signs of thyrotoxicosis, presumably because of insufficient absorption of thyroid hormone from the intestine. The following results were obtained from the four remaining dogs.

a. *Effect of thyroid hormone on the basal heart rate.* Each of the four animals showed an increase of approximately 30 to 60 beats per minute in the basal heart rate during the course of the feeding of thyroid substance. The average basal rate before thyroid feeding was 112 as compared with 161 after two to six weeks of feeding. It was more difficult to obtain basal heart rates in the latter case because of greater excitability of the animals and greater accelerator response to mild exercise. One of the animals was particularly well-trained, having been used for similar experiments under near-basal conditions for four years.

b. *Responses to continuous injection of adrenalin at low rates.* The average response of the normal dogs to a continuous intravenous injection of adrenalin, 1 part in 500,000, at a rate of 1 cc. per minute is illustrated in figure 1. The heart rate, after a latent period, was accelerated 17 beats per minute. This injection rate is recognized as being barely threshold for production of circulatory effects in the normal unanesthetized dog (21). The same injection rate in the same animals after 2 to 5 weeks of thyroid feeding resulted in a rate increase of 44 beats per minute over the already high basal rate. At the end of 75 seconds of injection of adrenalin an average rate of 124 beats per minute was obtained in the animals before

thyrotoxicosis as compared with a rate of 201 for the same animals after thyroid feeding.

Comparison of figures 1 and 2 will illustrate the differences in responses obtained when the adrenalin injection rate was doubled or quadrupled. Reflex inhibition of the heart as a result of the pressor effects of the higher dosage (fig. 2) becomes a factor but the reflex inhibition is less effective in opposing the direct cardio-accelerator action of the adrenalin in the thyro-

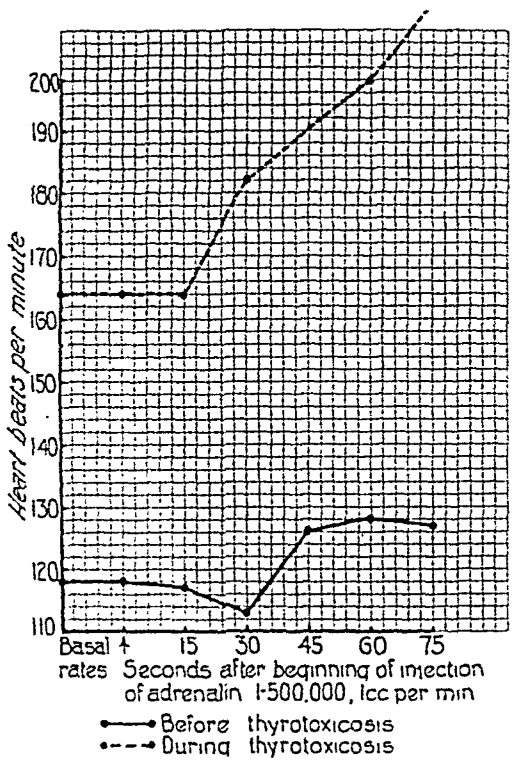

Fig. 1

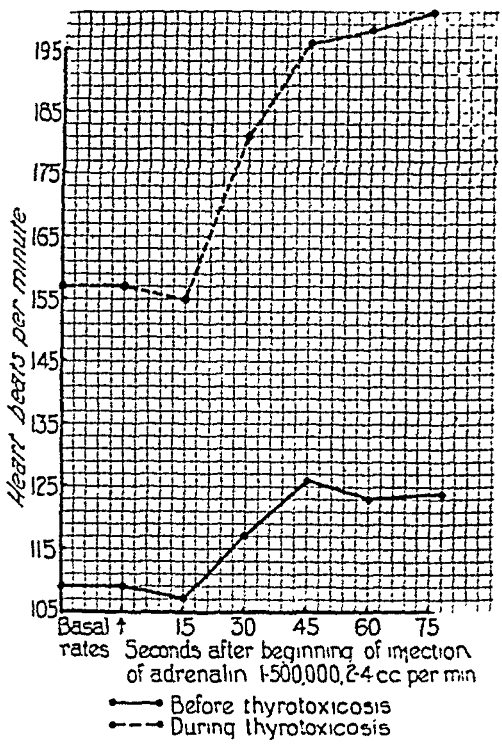

Fig. 2

Fig. 1. Average effect of continuous intravenous injection of adrenalin, 1 part in 500,000, at a rate of 1 cc. per minute on the heart rate of a series of dogs before and during thyrotoxicosis produced by feeding powdered thyroid.

Fig. 2. Average effect of continuous intravenous injection of adrenalin, 1 part in 500,000, at rates of 2 and 4 cc. per minute on the heart rate of a series of dogs before and during thyrotoxicosis.

toxic state than in the normal. It is to be expected that the pressor responses resulting from a given dose of adrenalin after thyrotoxicosis should be at least as great as in the same animals before thyrotoxicosis (2). Reflex inhibition of the heart quickly opposes the direct accelerator action of adrenalin, 1-500,000, injected at rates of 2 to 4 cc. per minute in the normal dogs so that the heart rate rises only 15 beats per minute above the basal rate, but the thyrotoxic hearts show an increase in rate to approximately 48 beats per minute above the high basal rate at the end of 75

seconds. If the pressor response to this amount of adrenalin during thyrotoxicosis is not less than before the thyroid feeding, the greater accelerator action of adrenalin in the thyrotoxic animals may be interpreted as indicating either sensitization of the cardio-accelerator mechanism or subnormal effectiveness of the cardio-inhibitory mechanisms. Heightened sensitivity to the accelerator action of adrenalin in isolated thyrotoxic hearts supports the interpretation that peripheral sensitization of the cardio-accelerator mechanism is, at least in part, responsible for the results obtained. Moreover, it has been reported (17) that thyroxin increases the effectiveness of the cardio-inhibitory mechanism.

2. *Effect of thyroid hormone on the sensitivity of intestinal smooth muscle (IA) to adrenalin, adrenine, and adrenergic neurohormones.* a. *Responses to injected adrenalin.* The five dogs were given 41 injections of adrenalin at rates ranging from slightly below the threshold intestine-inhibiting dose to doses four times as great. The records of intestinal motility were obtained from innervated Thiry fistulae in each of the animals. Simultaneous records were obtained from innervated and denervated Thiry fistulae in one of the animals. After having determined the sensitivity of the intestine of each normal animal to adrenalin, thyroid feeding was begun. A total of 47 adrenalin injections, duplicating the rates before thyroid feeding, were given the animals after the appearance of thyrotoxicosis was indicated by the general signs and the specific cardiac sensitization to adrenalin described above. Neither the innervated nor the denervated intestine of the thyrotoxic dogs showed any hypersensitivity to the inhibitory effects of adrenalin. The responses of the intestine of one of the dogs to adrenalin before and during the period of thyroid feeding is illustrated in figure 3. If thyroid hormone has any effect on the intestine-inhibiting potency of adrenalin it decreases it.

b. *Responses to reflexly liberated adrenine.* The brief fall in blood pressure caused by intravenous injection of a selected dose of acetylcholine into unanesthetized dogs produces a sharp fall in blood pressure which causes reflex liberation of adrenine from the adrenal medulla (22). The acetylcholine itself is quickly destroyed so that, after a few seconds, the effects of adrenine on denervated indicators may be observed. The adrenine-inhibition of the intestine produced following acetylcholine injection is no greater in the thyroid-fed dogs than in the same animals before feeding thyroid.

c. *Effect of thyrotoxicosis on the sensitivity of intestine-inhibiting reflexes.* Rectal stimulation reflexly activates inhibitory adrenergic nerves to the intestine causing, in sensitive unanesthetized dogs, complete inhibition of intestinal motility and entrance of sympathin into circulation (23). After a latent period a typical adrenalin effect is observed on the motility of a denervated intestinal segment in an adrenal demedullated, vagoto-

mized animal (23). Since the mediator of impulses at adrenergic nerve endings is apparently identical to adrenalin, in view of the facts presented in

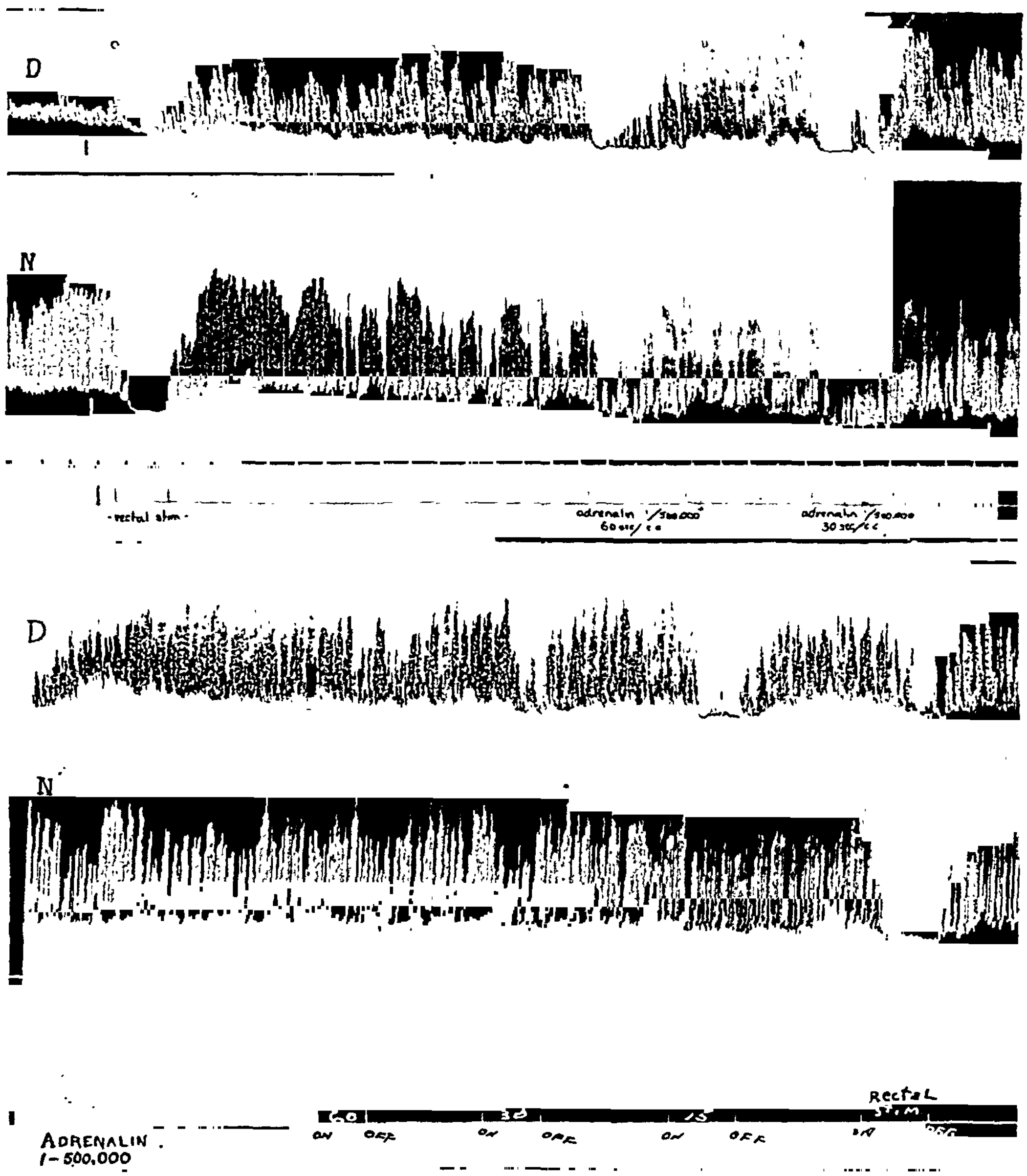

Fig. 3. *Upper record.* Effect on the motility of the denervated (D) and innervated (N) intestinal segments of rectal stimulation, injection of adrenalin, 1 part in 500,000, at a rate of 1 cc. per minute, and 2 cc. per minute. Time in minutes.
Lower record. Records from the intestinal segments of the same dog after four weeks of thyroid feeding. Effect of adrenalin, 1-500,000, at rates of 1, 2 and 4 cc. per minute, and rectal stimulation. Drum speed same as for upper record.

2 a, it is not to be expected that the recto-intestinal reflex would be sensitized during thyrotoxicosis unless the sensitization is elsewhere than

in the effector. Figure 3 illustrates the failure to increase the inhibitory effects of rectal stimulation in either the innervated or denervated intestine by thyroid feeding.

d. *Interpretations.* If alterations in the sensitivity of the intestine to adrenalin occur they are placed in evidence by near-threshold inhibitory injection rates. This method clearly depicts the development of hypersensitivity to adrenalin in the intestine after adrenergic denervation of the intestine (20). Therefore, the results presented in a to c indicate that the thyrotoxic intestine is not sensitized to injected adrenalin, reflexly liberated adrenine, the adrenergic mediator, or "intestinal" sympathin. The latter substances are similar, if not identical, to adrenalin. The absence of sensitization of the adrenergic intestine-inhibiting mechanism is further indicated by the fact that intestinal motility is not reduced by thyroid feeding. Sensitization of this mechanism would result in lowered motility of the intestine if the nerves are tonically active.

The question arises if it may be concluded that all smooth muscle having an IA innervation, whether in the intestine, bronchioles, bladder, nonpregnant uterus, or certain arterioles, is not sensitized to adrenalin by thyroid hormone. The necessity for a physiological classification of smooth muscle has been suggested (21). Such a classification is at present too incomplete to allow generalizations; however, it is likely that the intestine and other dually innervated smooth muscle having IA and EC innervation belong to the same physiological type.

SUMMARY AND CONCLUSIONS

A review of the literature on the relation of thyroid hormone to the sensitivity of autonomic neuro-effector systems reveals the fact that most observations have been concerned with excitatory adrenergic (EA) neuro-effector systems, such as the cardio-accelerator and vasoconstrictor mechanisms. Three other physiological types of autonomic neuro-effector systems, which have been given little attention, are inhibitory adrenergic (IA), excitatory cholinergic (EC), and inhibitory cholinergic (IC). Results obtained in the study of any one of these systems do not afford a basis for generalizations involving the other three.

An experimental analysis of the sensitivity of the cardio-accelerator mechanism (EA) and the intestine-inhibiting mechanism (IA) of dogs under near-basal conditions to adrenalin before and during thyrotoxicosis indicates that marked sensitization of the former mechanism occurs in the absence of any sensitization of the latter. The intestinal smooth muscle of the thyroid-fed animal is not hypersensitive to adrenalin or to adrenergic neurohormones, and there is no increased effectiveness of intestine-inhibiting reflexes utilizing adrenergic nerves.

Explanation of the thyrotoxicosis syndrome in the dog, in addition to the

usual recognition of the responses resulting from increased metabolism *per se* and direct effects of the thyroid hormone on effectors, requires recognition of *differential sensitization* of autonomic neuro-effector systems. The sensitization of these systems can not be stated in terms which refer to anatomical units of the visceral efferent system containing various physiological types of neuro-effector systems. Exactly which systems are sensitized and which are not must be determined by further experimentation.

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STUDIES ON THE RATES OF ABSORPTION OF WATER AND SALTS FROM THE ILEUM OF THE DOG

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It has been found by Roepke and Visscher (1939) that, during absorption from the dog's lower ileum of originally isotonic salt solutions, those solutions become significantly hypotonic as judged by vapor tension measurements. This phenomenon occurred when mixtures of NaCl and Na_2SO_4 in solution underwent absorption. If such solutions were originally slightly hypotonic they remained so, and if they were not too greatly hypertonic their osmotic activity decreased to less than that of the animal's blood. The same authors showed that when autogenous serum underwent absorption its apparent osmotic activity decreased to the extent of a half atmosphere osmotic pressure during the process. These observations point to a net absorption of solute in excess of its isotonic equivalent of solvent. The experiments using serum are particularly important because originally there are no concentration differences whatever, and any hydrolytic enzymatic processes occurring would increase rather than decrease osmotic activity. Studies have been made of the net movement of water and salt from various salt mixtures in order to ascertain the relation between the two. Such observations are reported in this communication.

From previous studies it has become apparent that the presence of polyvalent ions greatly influences the rate of uni-univalent salt absorption. No observations have as yet been reported, however, in which the rates of absorption from isotonic solutions containing various proportions of univalent and polyvalent ion salts have been studied under standardized conditions. In view of the difficulty of maintaining adequate constancy of conditions in repeated absorption studies in acute experiments under anesthesia, observations have been made upon dogs with chronic ileal loops. With suitable precautions repeated observations on such loops give constant results and comparisons can be made of the absorption rates under the several conditions.

Rates of water absorption over time are difficult of measurement by simple volume studies because it is impossible to empty the intestine completely several times during a single absorption period by feasible mechan-

ical means, and because the trauma incident to emptying affects the subsequent activity for a considerable period of time. Therefore repeated absorption periods of various total durations have been used, and the final volume measured by the reference constituent method, eliminating the errors due to incomplete emptying.

METHODS AND RESULTS. Chronic Thiry-Vella ileal loops, 40 cm. in original length, were prepared in dogs as described by Dennis (1939) except that the ends of the loops were tunnelled subcutaneously over the costal margin to facilitate closure by simple compression during observation periods. Out of a larger number studied, two animals showing the closest constancy of absorption rates under controlled conditions in repeated observations were selected for the main studies. All dogs were carefully trained to lie quietly on the observation table without restraint during periods of study. Prior to each experiment the loop was washed with 50 cc. 0.92 per cent NaCl solution at 38°C. introduced by means of a no. 14 French soft rubber catheter provided with multiple holes near the tip and inserted to the midportion of the loop. This fluid was removed with a gentle stream of air and 15 minutes allowed to elapse leaving the air-filled catheter in place before the absorption experiment was begun. All fluids were introduced and removed through the catheter which was emptied of fluid by introduction of 2 cc. of air during absorption periods.

Volumes of fluid in the loop were determined by the *reference constituent* method, employing either sulfate or chloride for that purpose. When sulfate-containing solutions were studied that amount of the fluid remaining in the gut at the end of a period of absorption which was readily withdrawn in a syringe connected to the catheter was so removed. Immediately thereafter a known volume of 0.92 per cent NaCl was flushed through the loop and collected. Both fluids were analyzed for sulfate and the true final volume calculated as the sum of the final fluid withdrawn by syringe plus the quantity,—wash volume times its sulfate concentration divided by the sulfate concentration in the final fluid. In using chloride as the reference constituent the procedure is the same in principle except that isotonic sucrose is used as the wash solution and calculations are made from chloride concentrations. By these methods volumes of fluid in loops can be measured with an accuracy of ± 0.4 cc. Water cannot be used as a wash fluid because, as Dennis (1939b) has shown, the ability of the intestine to absorb against gradients is impaired or temporarily abolished by short periods of exposure to pure water. Chloride was determined by the Van Slyke method and sulfate by the titrimetric benzidine procedure.

Experiments of two sorts have been performed and examples will be presented in this paper. In one case absorption over a given period of time (12 min.) was studied repeatedly on the same loop, using varying

proportions of two salts, NaCl and Na_2SO_4 in isotonic solutions in successive experiments at regular intervals. The period between trials definitely influences the results, absorption being slower when the interval is short.

TABLE 1

EXPERIMENT NUMBER	ORIGINAL CONCENTRATION		PERIOD OF ABSORPTION	FINAL CONCENTRATION		CALCULATED OSMOTIC EQUIVALENT OF NaCl AND Na_2SO_4 IN SOLUTIONS AS M. EQUIV. NaCl*	
	Cl	SO_4		Cl	SO_4	Initial	Final
	m. equiv.	*m. equiv.*	*minutes*	*m. equiv.*	*m. equiv.*		
a 1	156.0	0	10	112.0	0	156.0	112.0
2	141.3	25.2	12	74.5	58.0	167.2	111.1
3	131.0	42.0	12	59.0	98.0	157.5	120.8
4	79.0	126.0	12	11.0	203.4	158.4	139.1
5	43.6	202.0	12	1.6	254.0	170.9	161.7
b 1	156.0	0	12	125.0	0	156.0	125.0
2	141.3	25.2	12	109.5	26.8	167.2	116.4
3	79.0	126.0	12	33.7	160.0	158.4	134.5
4	43.6	202.0	12	16.3	218.0	170.9	153.6

* Calculated as the sum of chloride concentration plus 0.63 times that of sulfate in milliequivalents.

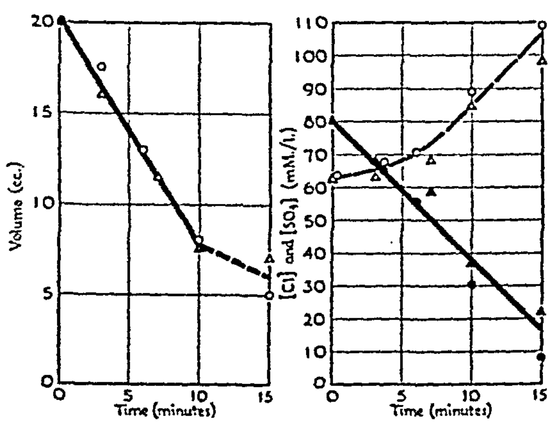

Fig. 1a Fig. 1b

Fig. 1a. The rate of volume decrease during absorption of an isotonic solution containing originally 63 mM Na_2SO_4 and 78 mM NaCl from a chronic Thiry-Vella ileal loop in the dog. For further details see text.

Fig. 1b. The changes in concentrations of $\bar{Cl}$ and $\bar{SO}_4$ over time in the experiments shown in figure 1a.

The composition of the fluids introduced in the experiments reported in figure 2 a and b is shown in columns 2 and 3 of table 1. In determining the rate of water absorption over shorter intervals of time the method used was in general the same except that the duration of absorption, rather than

the composition of the fluids was varied. Twenty cubic centimeters of solution containing 78 mM NaCl and 63 mM Na_2SO_4 were introduced into the loop and after a certain time interval withdrawn as described above.

The composite results of experiments upon the influence of the relative concentrations of sulfate and chloride in originally approximately isotonic mixtures of their sodium salts on the absorption process are shown in figure 1 a and b. The several rates measured are plotted against observed mean concentrations of sulfate and chloride during the period of absorption. The mean concentration over the time of absorption has been selected for comparison because original and final concentrations differ substantially from one another. The general significance of the results would not alter, however, if original concentrations were used for reference.

In figures 2a and b one sees that the absolute amount of chloride absorbed falls off as its mean concentration in the solution diminishes, but that the proportion of the total present which is absorbed increases with increasing sulfate concentration. This is evident from the course of the chloride clearance rate[1] curve. This calculation has no necessary connection with any particular mechanism of absorption. It is employed here simply as a measure of the rate of removal of chloride, in terms of cubic centimeters of fluid, *the chloride in which is removed from the gut, per unit of time.* It can be seen that the chloride clearance rate more than doubles on increasing the sulfate from 0 to 240 m. equivalents per liter.

Coincident with the decrease in absolute absorption of chloride with the higher concentrations of sulfate in the gut fluid, shown in the upper segments of figures 2 and 3, is an increase in absorbed sulfate. Our experiments on sulfate clearance are too few to allow a statement as to whether the relative absorption rate alters with concentration. This problem is much complicated by the fact that the degree of impermeability of the intestinal epithelium to sulfate is variable in different animals and under different conditions in the same animal. Poisoning or injury of other sorts makes the gut wall more permeable to sulfate.

The net rate of fluid absorption is also a function of the relative proportions of sulfate and chloride. However, over the range between 40 and 220 m. equiv. of sulfate there is by no means a direct proportionality in fluid moved. For a five-fold increase in sulfate concentration there is less than a 50 per cent decrease in net water movement, and over a considerable range of sulfate values there is no appreciable change in the fluid absorption rate. This is in spite of 100 per cent changes in chloride clearance over the same sulfate range. Thus although there was no more

[1] R_0 is the clearance rate for chloride. Its calculation is from the equation $\frac{CV}{C_0V_0} = \left(\frac{V}{V_0}\right)^{\frac{R_0}{D}}$ (Peters and Visscher, 1938).

net fluid transport, the fluid in the gut was nevertheless being cleared of chloride faster at sulfate values of 160 than at 40 m. equiv. This observation is extremely hard to harmonize with any theory of uni-univalent

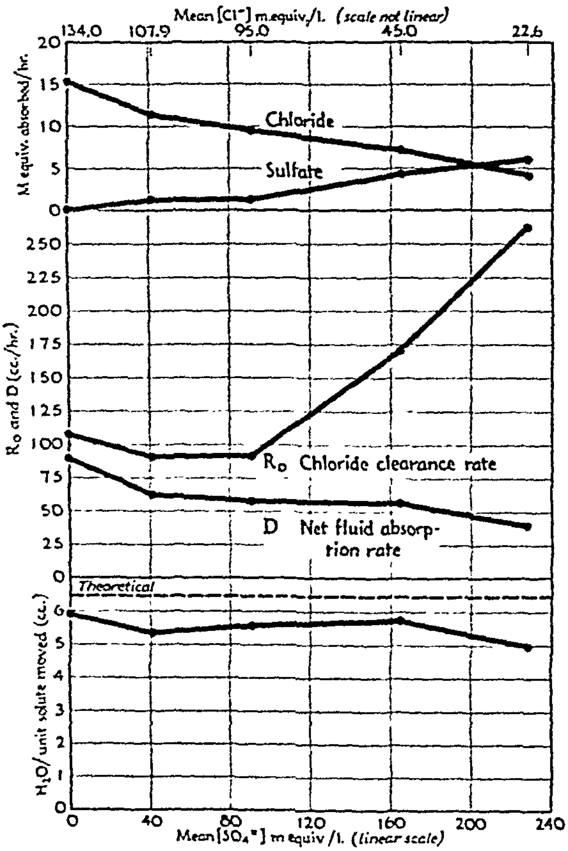

Fig. 2a

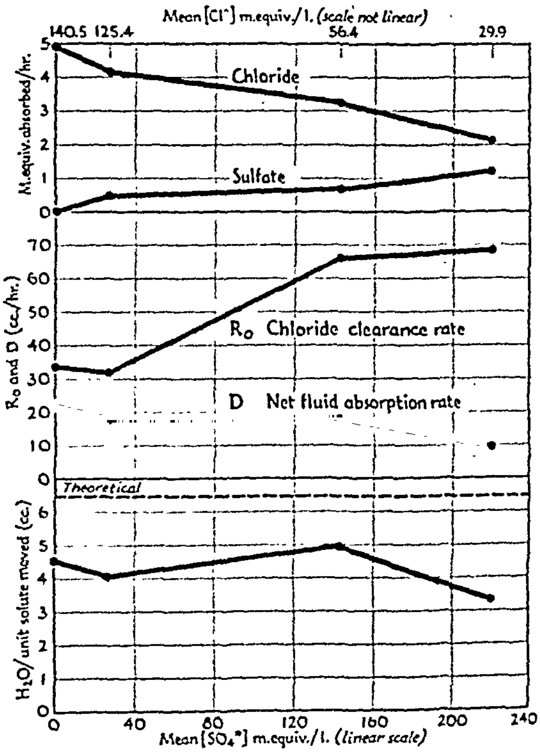

Fig. 2b

Fig. 2a. The rates of chloride and sulfate absorption (upper graph), clearance of intestinal fluid of chloride, R_0, adsorption of water, D, and solvent per unit of solute removed, from a chronic Thirty-Vella ileal loop in a one-year-old dog in relation to average concentration of NaCl and Na_2SO_4 in initially isotonic solutions placed in the loop. The values given on the abscissae are arithmetic mean concentrations determined from observed concentrations of Cl and SO_4 at the beginning and end of a 6 minute absorption period. Each set of vertical points represents a separate experiment on the same loop. In each experiment, except at zero concentration of SO_4 where a shorter period was used, absorption was studied over twelve minutes with samples drawn at six minutes. The volume change, D, was calculated for the twelve minute period, other values are for the second six minute period.

Fig. 2b. The same in the case of an ileal loop in a 6-year-old dog. Note the slower rates at all relative concentrations.

salt absorption which would involve one-way fluid movement related in any way to the salt movement. A fluid circuit theory is consistent with the facts.

The data available in these experiments allow one to calculate the

amount of water moved per osmotic equivalent of salt absorbed. This calculation acquires importance in view of the fact previously mentioned that these solutions become significantly hypotonic during absorption, as measured by vapor tension methods. Dog's blood is isosmotic with 156–162 mM NaCl. At the total ionic concentrations in question 1 mM Na_2SO_4 is the osmotic equivalent of 1.26 mM NaCl, according to vapor tension measurement. From these data one can calculate the osmotic equivalent of sulfate and chloride in terms of equivalent quantities of chloride alone. By dividing the figure for net water moved per unit of time by the osmotic equivalent of salt as NaCl moved, one obtains the observed value for water moved per osmotic equivalent of the two salts moved. The interesting result has come out of these calculations, shown in the lowest curves in figures 2a and b, that in no instance has the salt carried its isotonic equivalent of water with it from gut to blood, in terms of net water movement. The interpretation of this perfectly regular observation is not entirely obvious. Some solute undoubtedly enters the gut from the blood under the conditions in question (Peters and Visscher, 1938). However, as noted above, the gut fluid is hypotonic and therefore normal osmotic forces would be tending to move water from gut to blood. The main salt movement is of NaCl against its concentration gradient. It seems remarkable that this latter process outdistances the water movement. The fact that some solutes, such as urea and bicarbonate, may be entering the gut, the former in small and the latter in significant amounts (Lifson, 1940), does not clarify the question on this score because still one is confronted with the fact that during the process of absorption the gut fluid is significantly hypotonic in spite of the entrance of those substances. The simplest alternative or supplementary assumption which would account for the facts as observed would appear to be the hypothesis that a hypotonic solution entered the gut simultaneously with the exit of fluid.

Reference to table 1, columns 7 and 8, will show the further interesting fact that the calculated osmotic equivalent of the total NaCl and Na_2SO_4 in solutions in the gut after 10 to 12 minutes of absorption deviates from the isotonic equivalent of 159 mM NaCl to a greater extent the less Na_2SO_4 is present. These several facts are in apparent contradiction to the view that isotonic NaCl is absorbed by simple transport in isotonic solution.

CONCLUSIONS

1. A method is described for the measurement of water and solute absorption from intestinal loops, employing a reference substance.

2. Volume decrease during absorption is approximately linear, within the limits of accuracy of measurement, during the first 10 minutes.

3. The chloride clearance rate is a direct but non-linear function of the concentration of sulfate in originally isotonic solutions containing sodium salts of the two anions undergoing absorption.

4. The absolute absorption rates of both sulfate and chlorides in isotonic mixtures of their sodium salts are direct functions of their individual concentrations.

5. The net fluid absorption rate from isotonic solutions of varying proportions of NaCl and Na_2SO_4 is inversely related to the concentration of sulfate but not linearly so.

6. The net solute absorption is not equal to the calculated osmotic equivalent of water of the salt moved. The net effect is therefore an absorption of a hypertonic solution. As to mechanism the more probable reality is a two way movement in which a hypotonic solution enters the gut while salt solution leaves it to enter the blood. Intestinal transport may be looked upon as the algebraic sum of two-directional movements.

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THE SENSITIZATION OF THE DENERVATED HEART TO ADRENALINE

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Shortly after denervation certain structures of the body have been shown to become sensitized to chemical agents, natural or otherwise. Evidence for this "law of denervation" has been recently reviewed by Cannon and Rosenblueth (1937) and by Cannon (1939). For example, smooth and skeletal muscle, after being denervated, become more responsive than before to chemical stimulation. Heart muscle deprived of its nerves might be expected to respond in like manner. Sawyer, Hampel and Ring (1938), however, before using the denervated heart as an indicator in chronic experiments, tested it for sensitivity to adrenaline and drew the conclusion that it was not sensitized by denervation. The present paper deals with a further study of the response of the denervated heart to adrenaline.

METHOD. Healthy young cats were selected for each experiment. Under ether anesthesia the heart was surgically denervated according to the technique described by Cannon, Lewis and Britton (1926). The animals were then placed in warm cages for about 24 hours before being subjected to the first experiment. About 3 days after operation they began to eat, and within a week they were taking their food quite normally. They were allowed to convalesce 7 days before a second experiment was performed. Seven-day intervals separated subsequent experiments. Each animal showed a moderate loss of weight after the operation, with a gradual return to normal in about a month. Experimental results were not affected by weight variations.

For the experimental observations nembutal (0.7 cc. per kgm.), injected intraperitoneally as recommended by Hampel (1935), produced the proper depth and evenness of anesthesia during a period of 3 hours or more. At the conclusion of an experiment 60 cc. of warm normal salt solution was injected intraperitoneally, a treatment which seemed to have beneficial effects.

The anesthetized animal was placed on its side on an animal board, and the hind limbs were tied down in such manner that the femoral vein was readily accessible for exposure. The area over the vein was shaved and

cleansed with alcohol. A 25-gauge hypodermic needle, which had been soldered at right angles to the long axis of a copper rod, was inserted into the vein and held in place by a clamp. A continuous slow saline drip was then connected to the needle. It could be readily detached and temporarily replaced by a 1-cc. syringe.

The apparatus for recording the heart beat (Cannon, Lewis and Britton, 1926) consisted of a tambour which was placed between the animal board and the cat's chest. It was adjusted to the area of maximal cardiac impulse by packing cotton beneath it. The tambour was connected by rubber tubing to a sensitive Marey capsule the lever of which recorded the heart beats on a kymograph. A glass T-tube inserted into the rubber tubing made possible regulation of the pressure within the tambours and amplification of even poorly transmitted heart beats without compression of the chest wall. This fact is stressed because of marked changes in heart rate which may result from asphyxia. The interval of 24 hours between the operation and the first experiment was intended to avoid this possible complication, which sometimes resulted from post-operative pneumothorax.

At the beginning of each experiment a 10-γ solution of adrenaline was prepared from a 1:1,000 stock solution (Parke, Davis and Co.), with normal saline as a diluent. One cubic centimeter, containing from 0.05 γ to 10.0 γ of adrenaline, was injected into the femoral vein at an even rate in 10 seconds. Adrenaline deteriorization was carefully avoided. Solutions from 0.5 to 2 γ per cc. lost much or all of their effectiveness after standing for 20 minutes; in solutions containing 0.1 γ per cc. a similar loss occurred in half that time. Therefore, adrenaline solutions between 10 γ and 3.3 γ per cc. were not permitted to stand longer than 20 minutes; when the concentration was between 2 and 0.5 γ per cc. the drug was injected within 10 minutes after its dilution; and for doses of 0.25 γ or less not more than 5 minutes intervened before injection.

Twenty-five seconds before adrenaline was injected, a graphic record of the heart beat was started and was run for about 80 seconds. The heart rate for 10 seconds *previous to the injection and the maximal rate during* the response were counted.

The animals rested upon an electric heating pad throughout the test. Rectal temperature varied not more than 1°C. in any experiment. Within this range there appeared to be no correlation between temperature and heart-rate changes.

RESULTS. Doses of 0.05, 0.1, 0.25, 0.5, 1.0, 2.0, 3.33, 5.0 and 10.0 γ of adrenaline were used. The results occasionally obtained with the minimal dose (0.05 γ) may not be significant, since the increase over the basal rate was small and fell within the range of error of the methods employed. When amounts of adrenaline greater than 2 γ were administered, the denervated heart response in the same and in different animals varied

considerably, i.e., the percentile increase in the basal rate between the 8th and the 29th day as compared with that on the 1st day was either increased, unchanged or decreased. Moreover, in 3 animals cardiac arrhythmias developed in response to doses greater than 2 γ as early as 15 days after denervation.

Since results obtained with very small and with large doses of adrenaline were variable and unreliable, the data obtained with moderate doses

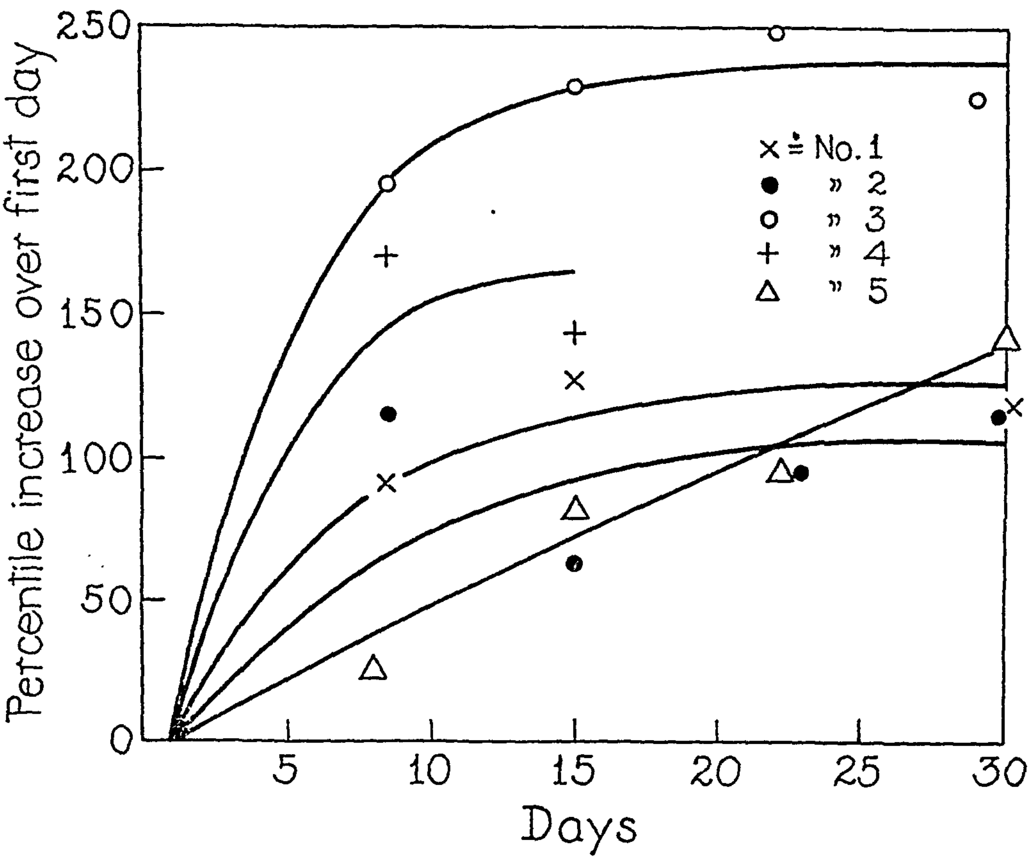

Fig. 1. Response of the denervated heart to 0.5 γ of adrenaline in 5 cats. The ordinates represent percentile increase in basal rate over that on the 1st day; the abscissae represent days after denervation.

(ranging between 0.1 and 2 γ) are presented as evidence for sensitization of the heart after being denervated.

For each dose from 0.1 up to and including 2 γ, the cardiac reactions to adrenaline fell into a general pattern. The percentile increase over the basal rate for a given dose rose rapidly between the 1st and 8th day after the denervation. After the 8th day the percentile increase over that of the 1st day either increased slowly or leveled off (fig. 1). One cat responded to 0.25 and 0.5 γ in an unusual manner in that the increases over the 1st day rose steadily for more than 3 weeks (cat 5, fig. 1). The responses to the other doses, however, were typical in this animal.

In table 1 the results for each dose of adrenaline are summarized in a representative animal. The percentile increases in heart-rate response over that on the 1st day are shown to vary from a minimum of 3 per cent on the 29th day with 2 γ to a maximum of 390 per cent on the 29th day with 0.1 γ (cat 2, table 1). It is also evident that the small doses of adrenaline give the greatest percentile increase over that on the 1st day. For example, on the 8th day the percentile increase for the 5 cats varied between 129 and 266 per cent with 0.1 γ and between 14 and 124 per cent with 2 γ. These results indicate that smaller doses of adrenaline, near the minimal, demonstrate most clearly the sensitizing effect of denervation.

TABLE 1

Cat 2. Response of denervated heart to adrenaline

ADRENALINE DOSAGE γ	Days after denervation of heart: 1			8				15				22				29			
	Heart rate per minute: Basal	Heart rate per minute: Maximum after adrenaline	Per cent increase over basal rate	Heart rate per minute: Basal	Heart rate per minute: Maximum after adrenaline	Per cent increase over basal rate	Per cent increase over 1st day	Heart rate per minute: Basal	Heart rate per minute: Maximum after adrenaline	Per cent increase over basal rate	Per cent increase over 1st day	Heart rate per minute: Basal	Heart rate per minute: Maximum after adrenaline	Per cent increase over basal rate	Per cent increase over 1st day	Heart rate per minute: Basal	Heart rate per minute: Maximum after adrenaline	Per cent increase over basal rate	Per cent increase over 1st day
0.1	119	121	1.7	135	142	5.2	203	148	157	6.1	258	152	164	7.9	365	133	144	8.3	390
0.25	127	135	6.3	146	163	11.7	85	169	185	9.5	51	165	191	15.7	149	158	180	14.0	122
0.5	124	140	12.9	144	184	27.8	116	168	204	21.4	63	167	209	25.2	95	151	193	27.8	115
1.0	118	144	22.0	142	203	43.0	95	152	208	37.0	68	163	237	45.5	107	137	196	43.0	95
2.0	129	178	38.0	144	228	58.2	53	173	243	40.5	8	164	248	54.3	43	164	228	39.1	3

After the 15th day of denervation, with doses of adrenaline over 1 γ, the heart in different animals exhibits either a decline or a rise in percentile increase in rate over that on the 1st day. In order to trace the development of sensitization to its completion, therefore, smaller doses than 1 γ must be used. In the present experiments the development of sensitization was followed for 29 days. The data do not indicate that the process even then had reached its conclusion.

The increase over the basal heart rate at successive 15-second intervals after the administration of 1 γ of adrenaline was analyzed on the 1st and on subsequent days after denervation. The percentile increase over the basal rate in one minute showed definite elevation from the 1st to the 15th day. Additional evidence of sensitization was also revealed as shown by a prolongation of the increase in rate on the 8th and on subsequent days as compared with that on the 1st day. For example, in cat 2 the heart rate

on the 1st day after denervation increased 4, 6, 4 and 3 beats at successive 15-second intervals after the injection of adrenaline; whereas on the 15th day it increased 4, 13, 12 and 10 beats, thus failing remarkably to decline to the basal rate at the end of 1 minute. Obviously adrenaline has a longer action on the sensitized heart. Moreover, the percentile increase over the basal rate is greater as sensitization develops if it is computed over 1-minute intervals (86 per cent in cat 2, on the 15th day, with 1 γ) instead of being computed on the basis of the maximal 10-second increase in rate (68 per cent). This is likewise due to a prolongation of adrenaline action on the denervated heart.

A manifestation of denervation other than heart-rate changes was noted and seems worthy of mention. The time interval between the injection of adrenaline and the maximal 10-second increase in heart rate was in every instance shorter on the 1st than on the succeeding days after the denervation. For example, in cat 4, with the injection of 1 γ of adrenaline, the maximal increase in rate occurred between 7 and 17 seconds on the 1st day, between 15 and 25 seconds on the 8th day, and between 17 and 27 seconds on the 15th day. There was, however, no close correlation between the length of the interval before the maximal increase of rate occurred and the duration of denervation or the dosage of adrenaline. The reason for the occurrence of this phenomenon is obscure.

Remarks. The results above reported disclose that within limits as time passes after denervation there is an increased heart rate in response to a given dose of adrenaline. The heart deprived of its nerve supply may therefore be considered to be sensitized to adrenaline. Justification for this assumption has been discussed by a number of authors (see Hampel, 1935). An explanation of the sensitizing of denervated structures is not revealed in the present experiments. The most plausible theory remains to be confirmed, i.e., that adrenaline and other substances act with more effectiveness because of an increase in permeability of cell membranes (Rosenblueth and Morison, 1934).

As previously noted, Sawyer, Hampel and Ring (1938) drew the conclusion that the heart is not sensitized to adrenaline by denervation. They used a method which differed from the present one in several respects: the cats were not anesthetized when adrenaline was injected, the adrenals were inactivated and the heart rate was not continuously recorded but was counted with a stethoscope. Nevertheless, their table, summarizing the results of a representative animal, shows upon careful analysis that, contrary to their inference, they actually demonstrated that denervation sensitizes to adrenaline. They injected 1 γ of adrenaline into the femoral vein 1, 2, 3, 4, 5, 9 and 12 days after denervation and counted the heart rate at successive 15-second intervals for 1 minute. If the increase in rate for 1 minute is counted on the 1st and the 12th days after denervation,

the percentile increase over the basal on the 12th day is found to be 60 per cent greater than that on the 1st day. An analysis of the present experiments using the same dose of adrenaline and identical methods of calculation not infrequently reveals a much higher percentile increase. In cat 2, the basal rate for 60 seconds increased to 86 per cent by the 15th day. Except two animals which showed increases of 52 and 56 per cent on the 15th and 8th day respectively, the percentile increase over the basal rate in 45 seconds ranged from 100 to 300 per cent from the 8th day of denervation onwards. That the percentile figures derived from the present experiments are higher than those obtained from the results reported by Sawyer, Hampel and Ring (1938) may be due to the differences in experimental procedures mentioned above. There is no doubt, however, that sensitization to adrenaline by denervation is revealed in both instances.

From the findings reported above, it is apparent that if the chronically denervated heart is to be used as a reliable indicator in experiments in which results are based upon variations in the response of the heart rate to adrenaline, the dosage and time after denervation must be carefully controlled.

SUMMARY

1. The denervated heart of 5 cats was tested for sensitization to adrenaline. The heart-rate response to graded doses of adrenaline (0.05 to 10.0 γ) was observed 1 day after denervation and every 7 days thereafter for 29 days.

2. Sensitization could not be reliably demonstrated with small (0.05 γ) and large (3.33 to 10.0 γ) doses of adrenaline. The larger doses frequently induced arrhythmias by the 15th day.

3. Definite sensitization always developed within 8 days after denervation (fig. 1).

4. A slight and progressive increase in sensitization usually occurred, with small doses of adrenaline, from the 8th to the 29th day following denervation, and there was no indication that sensitization was complete at 29 days (table 1).

5. After the 15th day of denervation it was necessary to use doses of less than 1 γ to detect further development of sensitization; doses near the minimal effective dose revealed it more clearly.

6. The response of the denervated heart to adrenaline was prolonged as denervation developed.

7. The latency of the maximal adrenaline effect on the 8th and subsequent days was increased over that on the 1st day.

8. The response of the denervated heart to adrenaline should not be used as an indicator in chronic experiments without first recognizing the existence of sensitization due to the denervation.

I wish to express my thanks to Dr. W. B. Cannon for suggesting this problem, and to Dr. R. Hodes and Dr. A. Ravin for their assistance and helpful advice.

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THE RELATION OF CONTRACTION OF DIFFERENT REGIONS OF THE VENTRICLE OF THE TURTLE TO THE RISE OF INTRAVENTRICULAR PRESSURE[1]

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In experiments recently reported from this laboratory (1) concerning the relation between electrical and mechanical events in the turtle's heart, local contractions of the surface musculature of the ventricle were recorded by means of a myograph simultaneously with potential time curves from the same region. The myograph, which operates by changing the resistance to an electrical current flowing through it, was described in detail in this communication. Since our report, the work of Shannon and Wiggers (2) on the isometric period of the ventricle of the turtle and frog has appeared. These investigators establish the presence of an isometric period in the sense of a rise of intraventricular pressure preceding by a definite interval the rise of aortic pressure. This finding led them to question the validity of myographic curves as indicating the onset of local muscle shortening. The work reported in the present communication is a further examination of this question by a study of the relations existing between myograms and pressure curves.

METHODS. For recording pressures a Gregg type of optical manometer, similar to that employed by Shannon and Wiggers was used.[2] To provide for greater convenience and flexibility, recording was made by the use of a photoelectric system instead of by the usual optical method. A light source, the manometer and a photoelectric cell were mounted together on a rigid metal frame which could be adjusted with respect to the heart so that only a short (10 cm.) lead tube was necessary for connecting the hypodermic needle (size 18) with the manometer. Heavy, tightly stretched rubber from an inner tube was used for membranes. The changes in current flowing through the photoelectric cell, due to changes in the amount of incident light reflected by the membrane mirror, were amplified by means of a direct current amplifier (3) and recorded by a cathode ray oscilloscope on bromide paper. For recording pressures from the ventricle and great

[1] Supported in part by a grant from the Wisconsin Research Foundation.

[2] We wish to acknowledge our indebtedness to Doctor Wiggers for the loan of a manometer for duplication and for invaluable criticism and advice in this work.

vessels, the light and electrical amplification were adjusted so that the movement of the cathode beam across the tube surface (a distance of 3 in.), corresponded to a change of pressure of about 30 mm./Hg. In a few experiments intra-auricular pressure was recorded simultaneously with myograms from the surface of the right auricle. The same membrane and manometer were used and the necessary sensitivity obtained by increase in the electrical amplification. Local shortening of the surface musculature from various regions on the ventricle were recorded by the myograph and a second direct current amplifier and oscilloscope simultaneously with intraventricular pressure. Mensuration of the curves was done with a micrometer comparator with 40x magnification (4).

Most of the experiments were done on large specimens of the snapping turtle (Chelydra serpentina). The large ventricle of this species made possible the recording of myograms from as many as 18 separate regions on the anterior surface. In several experiments the smaller turtle Chrysimus was used, and one experiment was performed on a large soft shell turtle (Amyda spinifer). In most cases artificial respiration was employed.

Results. The intraventricular pressure curve of the turtle shows an initial slow rise of pressure, amounting to several millimeters of mercury and lasting from 0.10 to 0.12 sec. In the measurement of these curves in the magnifying comparator, the horizontal hair of the microscope is adjusted to coincide with one edge of the curve and the carriage bearing the microscope moves with the micrometer screw until the edge of the curve leaves the hair. By this procedure, the onset of the initial slow rise of pressure may be determined quite accurately. The end of this period is indicated by a sudden increase of gradient, the pressure rising rapidly toward its maximum.

Myograms, registering the shortening of local regions of the ventricle, and recorded simultaneously with intraventricular pressure, show that at all surface regions, the onset of shortening occurs within the period of initial slow rise of pressure or follows the end of this period by a brief interval. Using the start of the rise of pressure as a reference for the myograms, the first region of the anterior surface of the ventricle to shorten is found to be the left base, and the onset of shortening in this region is coincident with the first detectable rise of intraventricular pressure. The apex and right base, which are the last regions to shorten, start to shorten late in the initial period of intraventricular pressure rise, or follow the end of this period by 0.01 to 0.02 sec. In other regions, the onset of shortening occurs at various times during the initial period of rise of pressure. The snapping turtle and Chrysimus showed the same sequence of involvement in the shortening process as was found in our previous work, in which a peak of a constant unipolar curve was used as a reference for the onset of shortening as recorded from various local regions by the myograph.

Figures 1 and 2 show the type of curves obtained from the ventricle. The upper curve in each record is the myogram, shortening of a local region of the ventricular surface being indicated by a downward movement. The lower curve records intraventricular pressure, a rise being indicated by an upstroke. In figure 1, the myograph was on the left ventricular base of a snapping turtle. The onset of shortening is coincident with the first detectable rise of intraventricular pressure. The change to an increased gradient in the pressure curve occurs 0.088 sec. later. The maximum intraventricular pressure is 26 mm./Hg, the heart rate 23 per min. Figure 2 is from the same heart and records the shortening of a region on the anterior midsurface of the ventricle. The onset of shortening follows the initial rise of intraventricular pressure by 0.027 sec., and precedes the onset of the sharp rise by 0.063 sec. The heart rate is 23 per min., the maximum pressure 25 mm./Hg. The speed of recording was 10 mm. per second in these two figures.

That local shortening of ventricular muscle occurs even though a change of volume of the chamber is absent, is illustrated in figure 3. Between the two cycles shown the great vessels were clamped. The result is a modification of the myogram in a manner indicating that full shortening no longer occurs, but the onset of shortening has the same time relation to the rise of intraventricular pressure as it did previous to the occlusion of the great vessels. *Before clamping, the maximum intraventricular pressure* was 25 mm./Hg. Clamping caused a marked increase. The record was made at a speed of 21.5 mm. per sec.

When intrapulmonic or intra-aortic pressure is recorded instead of intraventricular pressure, the onset of shortening of a local region of the ventricle precedes the rise of pressure by a longer interval as an expression of the isometric period. Figure 4 is an example. The upper curve records the shortening of a region at the left base of the ventricle of a snapping turtle. The lower curve is a record of intra-aortic pressure. The interval between the start of the descent of the myogram and the start of the rise of pressure is 0.29 sec. The record was made at a speed of 21.5 mm. per sec.

The results obtained from the two other species of turtles used were the same as those from the snapping turtle. Figures 5 and 6 are records, similar to those shown in figures 1 and 2, but obtained from the ventricles of Chrysimus and Amyda respectively. In figure 5, the myograph was on the left base. The onset of shortening is coincident with the initial rise of intraventricular pressure. The maximum pressure is 25 mm./Hg. In figure 6, the myograph was to the left of the mid ventricle. The onset of shortening follows the initial rise of pressure by 0.018 sec., and precedes the sharp rise by 0.063 sec. The maximum pressure is 30 mm./Hg. In both of these records the speed of recording was 21.5 mm. per sec.

The relation of local shortening of a region of the auricle to the rise of intra-auricular pressure is illustrated in figure 7. The myograph was on the middle of the anterior surface of the right auricle of a large snapping

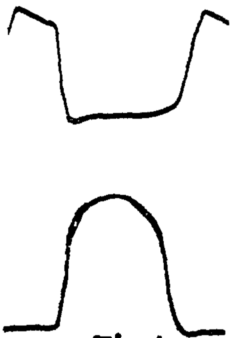
Fig. 1


Fig. 4

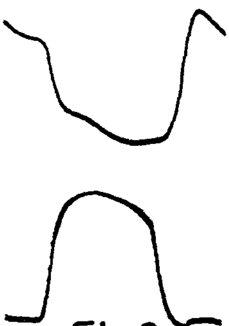
Fig. 2

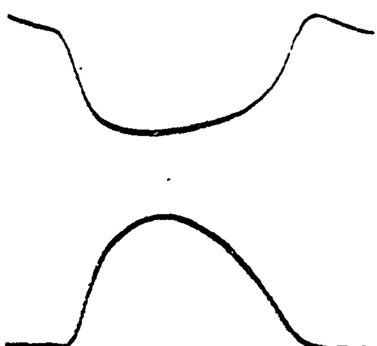
Fig. 5

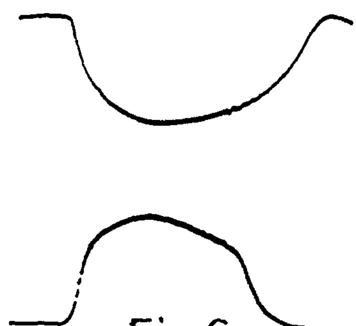
Fig. 6

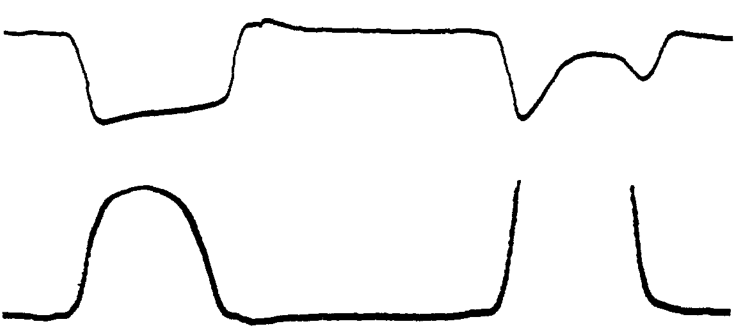
Fig. 3

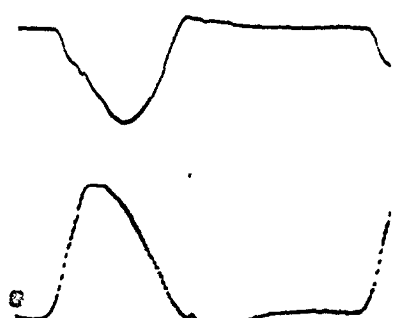
Fig. 7

Figs. 1–7

turtle. The onset of shortening follows the start of the rise of pressure by 0.05 sec. The total change of pressure is a little more than one millimeter of mercury. Speed of recording, 21.5 mm. per sec.

DISCUSSION. If it is assumed that the volume of blood in the turtle's ventricle is constant during the isometric period, the question arises as to how the shortening of the muscle can occur during this period. It is conceivable that with constant volume, local shortening might be made possible by local lengthening of the muscle in other regions. Against this interpretation is the fact that we have found no region in the normally beating ventricle in which the myogram shows any evidence of initial dilatation. It would appear that an adequate explanation of the shortening during the isometric period is present in the obvious change in shape that the ventricle undergoes during this period. The relaxed ventricle is approximately oval in shape. On contraction, the lateral walls are drawn in, the ventral-dorsal diameter increased, and the ventricle approaches the form of a sphere. This change in shape occurs early in the isometric period, and is probably in large part completed by the end of the initial slow rise of intraventricular pressure. This period has a duration of about 0.12 sec., and is associated with a rise of pressure of only a few millimeters of mercury. With the end of the initial period and the entrance of all or nearly all of the muscle in the shortening process, the intraventricular pressure rises rapidly to exceed the pressures in the great vessels and to end the isometric period. The earliest phase of systole of the ventricle is thus characterized by a marked change in shape of the chamber with little rise of pressure of the blood contained within it. Since the sphere has the smallest surface for a given volume, shortening of the muscle occurs without reduction of the volume of the blood in the ventricle.

That different parts of the dog's auricle enter into contraction at different times, has been shown by comparison of myograph and intra-auricular pressure curves (5) and by determination of the time relations between local shortening and electrical events (6). It has also been concluded, in large part from indirect evidence, that the same applies to the dog's ventricle (7). Wiggers introduced the term "fractionate contraction" to indicate the shortening of local muscle units (5). From the present report it is clear that this situation exists in the ventricle of the turtle.

CONCLUSIONS

The rise of pressure in the ventricle of the turtle during systole is characterized by an initial slow rise, amounting to a few millimeters of mercury and lasting about 0.12 of a second. This is followed by an abrupt increase of the pressure gradient. Local shortening of various regions on the anterior surface of the ventricle begin during the initial period of pressure rise or within an interval of several hundredths of a second after its termination. Shortening in all regions thus occurs before the pressure has risen sufficiently to force blood into the great vessels and reduce the volume of the ventricle. This shortening is made possible by change in

shape of the ventricle early in its contraction to approximate that of a sphere. Myograms from the surface of the ventricle of the turtle hence afford an adequate criterion for the onset of the local shortening process and for a comparison of electrical and mechanical events.

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ACETYLCHOLINE-EQUIVALENT CONTENT OF THE NASAL MUCOSA IN RABBITS AND CATS, BEFORE AND AFTER ADMINISTRATION OF ESTROGEN[1]

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There is both clinical and experimental evidence to show that the condition of the nasal mucosa is affected by sex hormones, particularly estrogen. The literature on this topic is both old and extensive (cf. Reynolds, 1939a; Hamblen, 1939; Fluhmann, 1939). In general, it may be said that when there is an abundance of estrogen in the blood, the nasal mucosa may be moderately hyperemic. Recently, Bernheimer and Soskin (1939) observed that while estrogen brings relief in some cases of atrophic rhinitis, it does not invariably do so; such patients, some of whom were women with normal menstrual (i.e., hormonic) cycles, were relieved, however, by topical application of prostigmine. These facts, along with other data which show that estrogen exerts within one hour a cholinergic action on the uterus of the rabbit (Reynolds, 1939b; Reynolds and Foster, 1939) suggest that estrogen may bring about in the nasal mucosa an increase in the concentration of free acetylcholine. This may result from increased production of the substance, or decreased destruction through diminished activity of cholinesterase.

In accordance with the foregoing considerations, the following experiments were carried out. These were planned to demonstrate the extent to which there may be a change in the concentration of an acetylcholine-like substance in the nasal mucosa following injection of estrogen. The method of tissue extraction and testing is that used heretofore by us (Reynolds, 1939b; Reynolds and Foster, 1939), and is that recommended by Chang and Gaddum (1933). The method is sensitive to 0.01–0.05 gamma of acetylcholine. Tissues from ovariectomized rabbits and cats were used. Castration was performed from six to seventy days prior to an experiment, although eighteen to twenty-one days was most often the elapsed time between operation and experiment. Cats were used in addition to rabbits since estrogen has no measurable effect on the concentration of acetylcholine in the uterus, as in the rabbit (Reynolds and Foster, 1940). The probable significance of this difference is discussed in the earlier paper.

[1] Supported by grants from the Josiah Macy, Jr. Foundation and the Committee for Research in Problems of Sex, National Research Council.

The experiments were carried out in the following way. On the day of an experiment, two rabbits were anesthetized by intramuscular injection of Dial (Ciba). One was used as an untreated control, the other received a subcutaneous injection of estrogen (Amniotin, Squibb; Progynon-B, Schering; Ben-Ovocylin, Ciba). Each of these estrogens exerts a cholinergic action on the uterus of the rabbit within an hour (Reynolds and Foster, 1939). The amounts used were 400 to 10,000 units of Amniotin, and from 0.05 to 2.0 mgm. of the estradiol compounds. When the animals were fully anesthetized, the skin over the nasal, frontal, and maxillary bones was removed. The nasal septum was cut across with a sharp scalpel and the nasal bones loosened laterally by means of bone forceps. They were then torn loose from their attachments by pulling dorsally with a hemostat on the nasal septum. In this way, the turbinate and ethmoid bones were exposed. The mucosa was then stripped off in as large pieces as possible. These were weighed immediately on a glass slide and immersed in 10 per cent tri-chloracetic acid. Extraction for an acetylcholine-like substance was then carried out. If an animal suffered from any obvious nasal congestion or infection, the tissue was not used. The quantities of tissue (mucosa plus some cartilage and superior turbinate bones) ranged from 0.5 to 1.5 grams per animal, although most often the quantity of tissue was about one gram. In every case, however, the amount of extract available was so small that it was impossible to employ the usual confirmatory tests for acetylcholine. Consequently, in the account which follows, we refer to the acetylcholine-equivalent content of the nasal mucosa, rather than to the acetylcholine content of the tissue.

RESULTS. *Activity of extracts from untreated animals.* In both cats and rabbits, the acetylcholine-equivalent content of the nasal mucosa is nil, or inappreciable in the majority of animals. This is shown by the following considerations.

It was observed that tissues from 59.1 per cent (13 of twenty-two) of the untreated rabbits and from 80 per cent (eight out of ten) untreated cats contained no measurable amount of an acetylcholine-like substance. Of the remaining 40.9 per cent of rabbits and 20 per cent of cats whose tissues yielded active extracts, the quantities were small; this is shown in figure 1, where the average concentration of an acetylcholine-like substance for each group is represented by the shaded bars, individual determinations from the several experiments, by dots. It will be seen that both the mean values and the range of individual points indicate a low concentration of an acetylcholine-like substance in the preponderance of cases.

Activity of extracts from estrogen-injected rabbits and cats. In contrast to the results of the foregoing group of experiments, the data from this group show that one hour after injection of estrogen, there is an appreciable

increase in concentration of an acetylcholine-like substance in the nasal mucosa. This was observed in both rabbits and cats.

The number of rabbits yielding measurably active extracts was increased to 75 per cent (eighteen out of twenty-four), the number of cats, from 20 per cent to about 90 per cent (ten of eleven cats). The mean concentrations and the data from individual experiments are shown in figure 1. In rabbits, the mean concentration was increased from 0.028 to 0.093 gamma per gram of tissue, or about a three and half-fold increase. In cats, it was increased from 0.018 to 0.094 gamma per gram of fresh tissue. These values are lower than those observed in uteri of rabbits, in which the effects of estrogen are intense (Reynolds, 1939b; Reynolds and Foster, 1939). In view of the lability of acetylcholine in tissues, however, and in

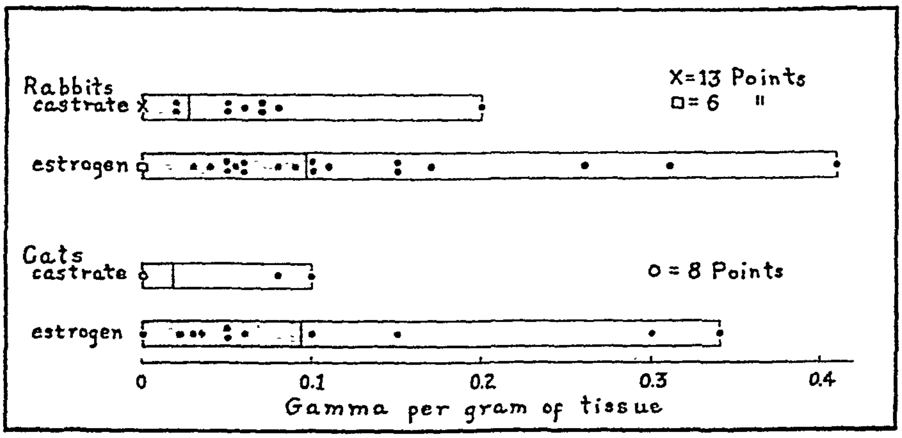

Fig. 1. Diagrams showing the mean concentrations of an acetylcholine-like substance (shaded bars) in extracts of nasal mucosa of ovariectomized rabbits and cats. Results from individual experiments are shown by dots. See text for discussion.

view of the small quantity and mixed character of the tissue used in the present work (mucosa, bone and cartilage), the results may properly be regarded as satisfactory proof of a cholinergic-like action of estrogen upon the nasal mucosa of both rabbits and cats.

A few experiments (five) were made with nasal mucosa from cats taken at the end of six hours after injection of estrogen. As has been found with the rabbit uterus, there is a diminution in the amount of an acetylcholine-like substance in the extracts. Two of the five were inactive, the remainder yielded concentrations of an acetylcholine-like substance of 0.04, 0.05 and 0.11 gamma per gram of fresh tissue, or an average for the group of 0.041 gamma per gram of fresh tissue.

SUMMARY

1. The effect of estrogen on the nasal mucosa is cited. It consists of moderate vasodilatation. Consideration of indirect evidence suggests

that it may be the result of a local increase in the quantity of free acetylcholine.

2. On this basis, comparison is made directly of the concentration of an acetylcholine-like substance in two groups of ovariectomized rabbits and cats, one of which is untreated, the other, after receiving a subcutaneous injection of estrogen.

3. It is found that both the number of extracts containing measurable amounts of an active substance, and the mean concentration of this material is increased one hour after injection of estrogen.

4. Attention is called to the fact that a similar cholinergic action of estrogen is demonstrable in the uterus of the rabbit, but not in the uterus of the cat. In the former, the uterine innervation is cholinergic; in the latter, it is adrenergic.

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EFFECT OF GELATINE FEEDING UPON THE STRENGTH AND FATIGABILITY OF RATS' SKELETAL MUSCLE

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Reports concerning the effect of gelatine upon fatigability, whether positive (Ray, Johnson and Taylor, 1939) or negative (Hellebrandt, Rork and Brogdon, 1940) have involved subjective criteria as to the point of fatigue. While such criteria may be valid for measurements of fatigue as a psychic reaction they do not necessarily reflect the functional capacity of the muscle. The object of the following experiments was to test, in an objective fashion, the effect of gelatine, fed as a supplement to an adequate diet, upon the functional ability of muscle.

TABLE 1

Effect of gelatine supplements upon skeletal muscle

SUPPLEMENT	NUMBER OF ANIMALS	TENSION-TIME	FATIGUE-TIME	MAXIMUM TENSION PER GRAM	CREATINE
		kgm. sec. per gram	*seconds*		*mgm. per 100 grams*
Gelatine	11	24.8	39	1,827	455
Glucose	9	24.2	39	1,761	450

Rats were used as the experimental animals. Adult males of four to six months of age from an inbred stock were matched as to size, and placed in two groups. Both were fed a stock diet of commercial dog biscuits. In addition, the individuals of one group were given ½ gram of gelatine in 3 cc. of water by stomach tube daily. The control group was similarly given ½ gram of glucose in 3 cc. of water. The supplements were continued for one week when the functional ability of one gastrocnemius of each of the rats was tested. For this, the animal was anesthetized with ether. The gastrocnemius was exposed and its tendon cut and attached to a torsion rod. The femur was rigidly fixed and the muscle stimulated directly with maximal induction shocks at the rate of 60 per second. The response was magnified with an optical lever and recorded on film. When the tension returned to the base line the stimulation was discontinued and the gastrocnemius removed from the animal,

weighed and analyzed for creatine. The tension curves were measured for 1, maximum strength; 2, the area of the tension curve to 10 per cent of maximum strength, and 3, the time to 10 per cent of the maximum strength.

The area of the tension curve, tension-time, has the same dimensions as work and is, therefore, proportional to the work capacity of the muscle. The data in the table show that there is no significant difference between the work capacities of the muscles of gelatine and glucose fed rats.

Fatigability is usually measured as the time from beginning of activity to the point of inability to respond. The time to complete lack of response is difficult to measure with accuracy. Therefore, the time required for the response to fall to 10 per cent of the maximum was measured. This fatigue time is identical for the two groups of rats.

The maximum strength of the two groups of muscles is not significantly different. This, taken with the similar areas for tension-time and identical fatigue times, indicates that the genesis of fatigue in the two groups is the same.

Analysis of the muscle showed, furthermore, that there were no changes in the average creatine concentration of the muscles of these two groups of animals.

The feeding time of one week was chosen as a period that should allow for such changes as reported by Ray, Johnson and Taylor, 1939. Because of their results the experiment was confined to male animals.

CONCLUSIONS

It may be concluded from these results that feeding of gelatine supplements to the normal adult male rat leads to no changes in the muscle itself which enhance functional performance.

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THE INFLUENCE OF MORPHINE ON TRANSPORTATION IN THE COLON OF THE DOG

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The correlation of normal activity and transportation in the colon of the unanesthetized, trained dog has already been studied (1). By letting the balloon serve as the bolus, it was possible to determine the relationship of transportation to the segments of the colon, periods of activity, sustained tonicity and even to the different phases of individual contractions. An efficiency gradient in which the greatest transporation occurs in the most proximal segments has been described as being dependent upon the character of contractions rather than upon the total quantity of activity. Transportation was found to be not only related to certain types of activity such as type II and III contractions but in the majority of instances to their systolic phases.

An extension of this work designed to study the force exerted by the colon in an attempt to move material to a lower level was measured by a technique as nearly isometric as possible (2). The pull pattern was found to be intimately related to the type II and III contractions and was only occasionally observed when the tone of the colon was extremely high and sustained for several minutes.

The effect of morphine on colon motility has been studied with the tandem balloon technique (3, 4). In these studies the augmenting effect of morphine upon the tone and activity of the colon was observed. The tremendous exaggeration of activity seen to follow the administration of moderate doses of morphine is not in accord with clinical observations that morphine is constipating, if the quantity of activity is to be considered the factor responsible for transportation in the colon. In other work (1, 2) it has been pointed out that transportation is more intimately associated with the character of activity rather than the quantity. Other observers have proposed that the constipating action is primarily related to an augmented sphincter activity which is far in excess of the augmentation occurring in other parts of the intestine. If this were the entire explanation one would expect a piling up of material in the best developed sphincter regions below the cardia, namely, at the pyloric, ileocecal and anal sphincters. However, while most observers are in accord in reporting

that this does occur at the pyloric sphincter no consistent findings have revealed an uneven distribution of material in other parts of the intestinal tract.

With the recording balloon used as a bolus free to move in the colon, the influence of morphine upon its movement was studied in a series of 15 experiments on 4 trained cecostomized dogs, following a series of 40 control experiments in which no morphine was used. The balloon bolus was inserted into the proximal colon by way of the cecostomy to a depth of 10 cm. beneath the skin and inflated according to a standard method (1). Before the injection of morphine 100 minutes of control motility and bolus transportation were recorded, thus making a total of 55 control periods of 100 minutes each (table 1). During the first and second 50 minutes of the 100 minute control period in 55 experiments the colon was active 48 to 56 per cent of the time and the balloon was observed to move approximately 4 cm. in each of the 50 minute divisions. During the first 50

TABLE 1

Effect of morphine on transportation

50 MINUTE PERIODS	1	2	3	4
*Minutes of control motility	25	28	27	27
Centimeters of control transportation	4.1	3.9	3.6	2.6
Minutes of motility after morphine			42	46
Centimeters of transportation after morphine			17	0.2

* Fifty-five experiments—40 = continued as controls; 15 = morphine injected at close of 2nd 50-minute period.

minutes following the injection of morphine the activity of the colon segment occupied by the balloon was found to be increased to 84 per cent and transportation to 17 cm., while the control tracings for this same period in 40 experiments reveal an expectancy of activity to be 51 per cent and a transportation of only 3.6 cm. During the second 50 minute period which followed the injection of morphine the activity of the colon was higher (92 per cent) than that of the first 50 minutes, but the transportation was practically negligible, amounting only to 0.2 cm. during the entire period. These figures are in marked contrast to the transportation expectancy for this period suggested by the control experiments.

To study further the effect of morphine on the colon 15 experiments were conducted in which the force exerted by the colon to displace a bolus to a lower level was measured by an isometric apparatus previously described (2). From the data thus obtained it was evident that here too the character of activity is more important than quantity of activity. Simultaneous with the increase in activity elicited by morphine there was recorded an increase in transportation force. When the activity was se-

tained either as high tone or exaggerated type I and type II contractions, as was the case in the majority of instances, the force tending to displace the balloon rapidly diminished. On the other hand, in those instances where high tone or type III contractions alternated with brief periods of relative quiet or exaggerated type II contractions the tendency to displace the balloon was more evenly distributed throughout the experiment and more conspicuously associated with rising tone or type III contractions.

These findings corroborate previous work (2) which has associated the pull pattern with types of activity and together with the data on transportation suggest that the tendency of the colon to transport material from one segment to another is dependent upon the type of activity in adjacent segments as well as the type of activity in the segment in which the bolus rests.

The tandem balloon technique has revealed certain periods after the injection of morphine in which the different segments of the colon are not in simultaneous activity. The character of activity at this time is very comparable to that of the activity recorded by the single balloon during the time when transportation and transportation force is being manifest. It is logical that constriction of any segment of the colon tends to prevent the passage of material through its confines from an adjacent segment even though it tends to empty itself of its own contents. It seems reasonable therefore that a segment of high pressure will be able to empty itself only if an adjacent segment is of lower pressure. The efficiency gradient (1) is therefore at least in part dependent upon the state of activity existing in adjacent segments. Normally this efficiency gradient tends to permit material to pass through the colon at a physiological rate. The injection of morphine augments the normal gradient immediately following its administration by exerting its first effects most profoundly upon the more proximal segments. Shortly after this immediate effect the extent of the morphine action is more evenly distributed to all parts of the colon, making each segment serve as a barrier to the passage of material through its limits. Thus all parts of the colon become physiological sphincters retarding the shifting of materials. This conception merely expands upon an older conception that augmented sphincter action at the anatomical sphincters accounts for the constipation following morphine administration.

It was found early in the work with the tandem balloon system that the colon of the dog may be divided into two physiological units not dependent upon anatomical divisions (5). These units, proximal and distal segments, are characterized by certain types of activity but the dividing line between them may shift from time to time. This division although usually in the region of the splenic flexure might shift to any part of the colon or might

disappear entirely, permitting the colon to act in its entirety as a proximal-like or distal-like unit. Since the proximal segment is characterized by pressure changes of the type II and type III variety directed in an aboral or oral manner and the distal segment is characterized mainly by type II simultaneous contractions, material is caused to shift more rapidly in the proximal than in the distal segments. This probably is a factor in explaining the well known clinical observations that the distal colon is more responsible for constipation than is the proximal. The character of activity observed in the second phase following morphine injection (retarded efficiency gradient) is more like that of distal colon activity in that pressure changes with directional character are largely replaced by strong type I and II activity of stationary character.

The authors are indebted to Dr. A. J. Carlson, who made this study possible.

SUMMARY

1. Following the administration of moderate doses of morphine to unanesthetized dogs, there was an immediate augmentation followed by a retardation of the rate of transportation of a bolus in the colon.

2. The force exerted by the colon to transport a bolus was measured by an isometric technique which revealed a close correlation between that force and the character of activity rather than the quantity of activity.

3. A dual effect of morphine is described as being first an augmentation and second a retardation of the normal "*efficiency gradient*."

4. The phase of retardation in the normal "*efficiency gradient*" in which activity in the lower segments more nearly approaches that of the proximal segments in extent and character may account for constipation after morphine administration.

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THE DEVELOPMENT OF THE ANACROTIC AND TARDUS PULSE OF AORTIC STENSIS[1]

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Cardiac specialists have long possessed the empirical knowledge that marked stenosis of the aortic orifice is accompanied by characteristic changes in the radial pulse. There is a definite halt in the rise of pressure and the principal peak occurs perceptibly later in the cycle than it does in the normal pulse. These abnormalities can be shown in a radial sphygmogram, and in severe cases can be detected by expert palpation. Attempts to explain the hydrodynamic mechanisms underlying the association of these phenomena have stopped short of completeness. Katz, Ralli and Cheer (4) showed the changes in the central pulse produced in dogs by gradually tightening a ligature around the root of the aorta. Emphasis was laid upon a deep notch developing, with increasing stenosis, on the anacrotic limb as the probable source of the anacrotic halt in the radial pulse. Feil and Katz (1) presented paired simultaneous sphygmograms from subclavian and radial arteries of stenosis patients, showing associated abnormalities in the two pulses. No one has heretofore bound the two researches together with a study of the development of the peripheral from the central pulse during progressive stenosis of the aortic orifice. This report presents the results of experiments designed to fill that gap.

Method. Dogs were anesthetized with sodium barbital (250 mgm. per kgm. intravenously) after a small dose of morphine. Under artificial respiration the chest was opened wide in the mid-line and the heart was suspended in a cradle made of the opened pericardium. A stout loop (a round shoe lace) was passed around the root of the aorta and fastened to a device with a calibrated screw, with which any desired degree of stenosis could be obtained and reproduced (used by Katz, Ralli and Cheer). The central pressure pulse was obtained by the use of a six-inch cannula inserted into the left common carotid artery and pushed down until its tip was in the ascending aorta just distal to the ligature. This cannula, like all the rest, was connected by lead tubing to a high-frequency ma-

[1] Preliminary report, This Journal 129: P 347, 1940.

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nometer of the Gregg type, the rubber-membrane modification of the Hamilton manometer, recording photographically at a distance of about two meters.

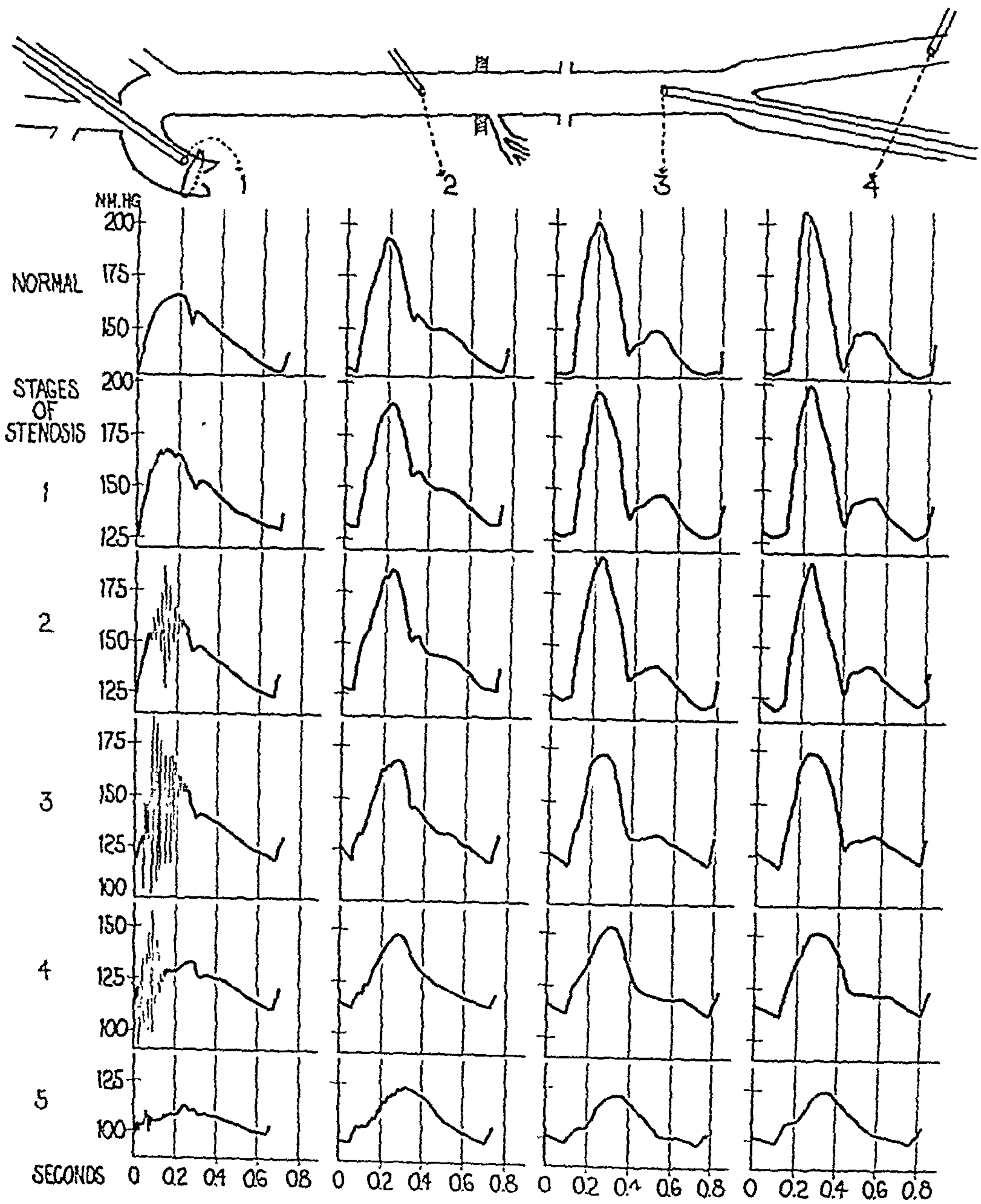

Fig. 1. Pressure pulses recorded simultaneously (each horizontal row) from four different parts of the arterial system with gradually increasing aortic stenosis (reading downward). Note that in each vertical column zero time indicates the start of the pulse in the ascending aorta.

In several experiments, various peripheral pressure pulses were obtained by the method of Hamilton and Dow (3), using a long cannula run up from

the right femoral with its tip movable to any desired point in the descending aorta-iliac-femoral system. At each of several positions of this cannula, a series of paired simultaneous pressure pulses was obtained for each of ten or more degrees of stenosis. While much valuable information was obtained by this method, it was sometimes difficult to compare the results quantitatively because of small changes in the central pulse from one series to the next. Therefore, in the experiment reported here, the tip of the long cannula was left just above the bifurcation, the aorta was punctured with a needle cannula just above the diaphragm, and a needle was inserted in the left femoral artery. This arrangement furnished four simultaneous pressure pulses from different parts of the system at each stage of the stenosis.

RESULTS. Reconstructions, to the same time and pressure scales, of a representative series of records are shown in the accompanying figure. Each horizontal row depicts the pressure pulses obtained simultaneously at the four designated arterial points at the indicated stage of stenosis. The following features of these pressure curves may be pointed out as pertinent to the problem.

The findings of Katz, Ralli, and Cheer on the changes in the pulse at the root of the aorta with stenosis are confirmed. Most obvious is the production of a mid-systolic turbulence when the orifice is narrowed partially but not enough to diminish the discharge. The breakdown of the smooth ejection into turbulence is signaled, as in their records, by a sharp anacrotic notch. As stenosis becomes more nearly complete, the systolic discharge is severely cut down, with the result that after the third stage the turbulence diminishes and finally disappears, and systolic, diastolic, and pulse pressures all descrease markedly. Less obvious at first glance but easily measurable is a prolongation of systole with no change or even a slight increase in the heart rate. The peak of this aortic pulse occurs a little later than in the control, but only in proportion to the systolic lengthening. The incisura is flattened almost to extinction.

It should be observed that two marked changes take place in the fundamental form of the central pulse: the initial rise becomes progressively lower and less violent, and it is followed by a short plateau merging into a gradual ascent to the peak.

The most striking change in the form of the other pulses (columns 2, 3, 4) is a progressive decrease in the amount of modification with transmission. The four pulses are quite different when there is no stenosis, but they become more like each other and like the central pulse through a gradual collapse of a high peak early in the cycle. It should be noted that except for a few cases in the thoracic aorta they are all smooth and show no transmission of the vibrations of the central turbulence.

DISCUSSION. The only concept, essential for the conversion of the

foregoing observations into an explanation of the radial pulse of aortic stenosis, is Otto Frank's (2) likening of the peripheral pulse to a slow manometer's recording of the central pulse. Such a manometer distorts both form and size of rapid pressure fluctuations because of the inertia of the fluid and its momentum when set into periodic oscillations. The degree of distortion depends upon the magnitude of pressure changes which are too rapid to be followed faithfully.

In the above experiments it can be seen that the stenosis restrains the impact of the usual sudden ejection into the aorta to such an extent that the peripheral pulse is permitted to reproduce quite faithfully the fundamental form of the central pulse. The resistance to flow at the stenosis has meanwhile molded the central pulse into just the form actually found at the radial in clinical cases.

The tardus quality of the stenotic pulse can be seen in the figures to be due to the disappearance of an early peak rather than to the introduction of a late one. Hamilton and Dow's (3) identification of the principal peak of the femoral pulse as a standing wave rested upon the determination that it occurred simultaneously everywhere beyond a point of phase reversal near the aortic arch. The peripheral pulse, therefore, starting later, shows its principal peak sooner after its start than does a central pulse. When the ejection is not sufficiently violent to set up this standing wave, the remaining later peak is merely the representation of that in the central pulse, formerly obscured by the superimposed wave. This disappearance can be followed, reading downward in each column of the figure, until only the fundamental central pulse form is left, modified on the anacrotic limb by the restraint which the stenosis imposes on the ejection.

In an unpublished experiment (shown before the American Physiological Society at Washington, 1936) Dow and Hamilton demonstrated the importance of the factors mentioned above. In a dog's aorta, in situ but completely tied off, relatively slow pressure changes, even though large, impressed at the cardiac end, were reproduced faithfully at the bifurcation. Even small sharp impacts, on the contrary, were distorted in form and magnitude by the periodic oscillations or standing waves set up.

This analysis agrees in many ways with that of Feil and Katz. It departs from that of Katz, Ralli and Cheer in minimizing the importance of the sharp downward break in the central pulse at the start of the turbulence as the source of the anacrotic halt in the radial pulse. In these experiments the two phenomena seem quite independent. The "anacrotic incisura," caused by the sudden breakdown of smooth flow into turbulence, reaches a maximum with moderate stenosis, then decreases in size; while the anacrotic plateau, due to interference with ejection, continues to grow in prominence.

It is true that the present experiments do not correspond in detail to the clinical picture explained by them. The development of the femoral rather than the radial pulse has been chosen for its accessibility. However, the same dynamic factors are operative in both cases and any difference can be only a quantitative and not an essential one. As was pointed out by Katz, Ralli, and Cheer, the artificial stenosis beyond the valves rather than at them permits better maintenance of the coronary flow than is possible in the clinical case. Perhaps this feature compensates for the rapid induction of the stenosis with no opportunity for other mechanisms to become useful.

Consideration of the simultaneous pulse in the thoracic aorta suggests that the diminution of pulse pressure in the ascending aorta may be somewhat exaggerated by recording the central pulse in the turbulent stream right at the orifice. Although the cannula pointing toward the heart should pick up the total pressure (lateral plus velocity), it is possible that the tip may have been pushed to one side, out of the direct stream and into an eddy pocket.

SUMMARY

The anacrotic halt and tardus characteristics of the radial pulse in cases of aortic stenosis are shown to be due to the following factors:

1. Stenosis so reduces the violence of the systolic discharge that standing waves are not set up and the peripheral pulse reproduces the central pulse form with almost complete faithfulness.

2. The stenosis offers so much resistance to flow during mid-systole that the central pulse itself assumes the anacrotic and tardus characteristics.

Acknowledgment is gratefully given to Dr. Carl J. Wiggers for the suggestion of this problem and for kindly advice and assistance. Thanks are also due to Messrs. J. H. Geyer and B. S. Brown for technical aid in several experiments.

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GRADED PARTIAL PANCREATECTOMY

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Ceccherelli (1) cited the early work of Brunner in 1682 as first demon strating that partial pancreatectomy did not impair the health or digestion of the experimental animal. Total pancreatectomy, however, is incompatible with life unless some form of substitution therapy is employed.

In 1939 we (2) reported from this laboratory a series of experiments demonstrating that in dogs in which a large portion of pancreas had been removed, degenerative changes in the liver could be prevented if the transected end of the remaining pancreas was transplanted into the stomach, thus preserving its external secretion. It has been observed repeatedly by numerous investigators that partial pancreatectomy does not disturb, to any degree, the sugar metabolism. We have interested ourselves in attempting to determine how much of the pancreas could be removed before the health of the animal was impaired as determined by blood sugar, blood amylase, plasma prothrombin determinations and microscopical studies of liver biopsies.

METHOD. Healthy mongrel dogs of both sexes, weighing between 6 and 15 kgm., were used in this experiment. They were kept on a diet of milk and bread, supplemented with Karo, yeast and cod liver oil.[1] After varying control periods, during which determinations of the blood sugar, blood amylase and plasma prothrombin were made, partial pancreatectomy was performed. Varying amounts of the gland were removed. At the time of operation, a liver biopsy was taken, and this repeated at weekly intervals for the first postoperative month. At approximately 60 and 90 days postoperative, other liver biopsies were taken, and again at postmortem examination. The values of the blood sugar, blood amylase and plasma prothrombin were determined at the time the liver biopsies were taken, or in some animals, more frequently during the first week after operation.

The blood sugar was determined by Benedict's (3) method. The blood

[1] Milk and bread in the ratio of 4 quarts to 3 loaves. Karo—3 tablespoons to each quart of the above mixture. Yeast—3 teaspoons to each quart of the above mixture. Cod liver oil—1 tablespoon to each quart of the above mixture.

amylase was done by a slight modification of the procedure described by Elman (4). To 5 cc. of a colloidal suspension of 2 per cent soluble starch, 1 cc. of plasma and 2 cc. of 0.9 per cent sodium chloride were added. This was done in duplicate for each blood specimen. On one of the resulting mixtures a sugar determination was run *immediately* (without filtration). The other was incubated for 30 minutes at 37.5°C. before the sugar content was determined. The difference between the non-incubated and incubated specimens represents the amylase value in terms of the milligram per cent sugar produced by the action of the serum amylase on the starch solution. The sugar in the non-incubated specimen was found to be slightly higher than the combined values of the blood sugar and starch blank.

The plasma prothrombin was done by the method described by Warner, Brinkhous and Smith (5).

At the time of death, the remnant of pancreas was weighed, and from this figure was calculated the per cent of the gland which had been removed.

RESULTS. The gland remnants were weighed at the time of postmortem examination. It was found that a rather regularly graduated series had been obtained in which the amount of the pancreas removed varied from 17 to 84 per cent. The individual proportions of removed tissue were 17, 19, 24, 48, 53, 63, 66, 71, 73 and 84 per cent.

Sugar. In none of the dogs was diabetes produced, except in the animal from whom 84 per cent of the pancreas was removed. In all the others, the blood sugar values did not vary beyond the normal range. The animal which developed diabetes died two weeks after operation. The blood sugar rose to 273 mgm. per cent one week after operation and reached 426 mgm. per cent three days before death. The blood sugar taken on the ninth postoperative day showing 102 mgm. per cent came after a three day fast.

Amylase. The blood amylase values varied over a wide range both during the control and postoperative periods. In each animal the fluctuation was marked. For convenience of statistical analysis, three groups were formed. In the first group were the animals from whom 17, 19 and 24 per cent of the pancreas had been removed. In the second group were those animals who lost 48, 55, 63 and 66 per cent of the pancreas at operation; and in the third group those from whom 71, 73 and 84 per cent of the gland had been removed. The amylase values were analyzed preoperatively and postoperatively within each of these groups.

It is evident that the difference in pre- and postoperative means (table 1) is well below the minimum difference necessary for significance. The same is true when the postoperative means of the various groups are compared. These figures seem to indicate that the removal of varying pro-

portions of the pancreas, up to 84 per cent, had no significant effect on the blood amylase level.

Prothrombin. The plasma prothrombin was not significantly altered by the removal of varying portions of the pancreas. The greatest variation was from 80 to 100 per cent, but this occurred in only one dog (no. 8). In four others, the variation was from 86 to 100 per cent, while in the remainder it was between 90 and 100 per cent.

Liver biopsy. The liver biopsies removed at weekly and later monthly intervals after operation showed no conspicuous change from the normal. In the animal with 66 per cent of the pancreas removed (no. 7), the sections of liver removed on the twenty-first and twenty-eighth postoperative days showed slight fatty degeneration, which, however, was not evident in the sections removed 59 and 118 days postoperative. The only other evidence of fatty change in the liver was found in the tissue removed from dog 8 (71 per cent of pancreas removed) on the thirteenth postoperative day. None of the sections of liver from the animal with 84 per cent of the pancreas removed showed any fatty degeneration.

TABLE 1

Statistical data for amylase values in different groups

	MEAN	S.D.	P.E.	MINIMUM DIFFERENCE FOR SIGNIFICANCE	ACTUAL DIFFERENCE
Group I					
Preoperative	529	172	41	147	31
Postoperative	560	152.5	21		
Group II					
Preoperative	567	159	27.6	108	74
Postoperative	493	159	19.6		
Group III					
Preoperative	570	171	34.6	138	75
Postoperative	495	164	25.5		

S.D., standard deviation; P.E., probable error.

SUMMARY

1. Portions of the pancreas, varying from 17 to 84 per cent, were removed from ten dogs, and their courses followed with blood sugar, blood amylase and plasma prothrombin determinations. Liver biopsies were examined at definite intervals postoperatively.

2. Only in the animal from whom 84 per cent of the pancreas had been removed did diabetes develop. All other blood sugar values were within normal limits.

3. Blood amylase values varied over a wide range both pre- and postoperatively. Statistical analysis of the data reveals no significant change in blood amylase values caused by removal of from 17 to 84 per cent of the pancreas.

4. Plasma prothrombin values were not affected by partial pancreatectomy.

5. The liver biopsies showed, in general, no conspicuous change from the normal. Fatty degeneration was present transiently in the two animals from whom 66 and 71 per cent of the pancreas had been removed. In none of the other biopsies was fatty degeneration seen.

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HORMONAL FACTORS AFFECTING THE SURVIVAL OF ADRENALECTOMIZED MICE[1]

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Synthetic progesterone and the hormones from ovaries heavily luteinized by pretreatment with gonadotropins have been shown to ameliorate the symptoms of adrenal insufficiency in rats (1–7), cats (8, 9), and ferrets (10), whereas male hormone is ineffective in this respect (4) or even harmful (11). If a similar differential effect of these substances obtains in mice, adrenalectomy should constitute a technique for distinguishing between large amounts of endogenously produced progesterone and androgen as, for example, following prolonged treatment of female mice with large doses of pregnant mare serum (12). This procedure cannot at present be utilized because of the paucity of information available concerning the effects of the different sex hormones upon the adrenalectomized mouse. With the object both of acquiring this needed information and of determining the effects of other sex hormones as well as of an adrenal cortical hormone in a species thus far largely neglected in this respect, the present study was undertaken.

Material and methods. A total of 195 adrenalectomized mice of the A (Strong) strain were used in these experiments. Adrenalectomy was performed as a single stage operation, using the bilateral lumbar approach with a single dorsal skin incision. The adrenal vessels were not ligated, but instead were pinched off well behind the adrenal with a small curved forceps in such a manner that the adrenal was removed with the surrounding fat. To rule out the factor of personal differences in operative technique, all the adrenalectomies were performed by the same operator. Castration was performed by the usual techniques. In all the operations the animals were anesthetized with ether. When both castration and adrenalectomy were performed, a period of at least 10 days intervened between the two operations.

[1] Supported in part by the Rockefeller Foundation and the Fluid Research Fund of Yale University School of Medicine.

The synthetic hormones[2] used in this investigation were estradiol benzoate, testosterone propionate, progesterone and desoxycorticosterone acetate. These hormones were dissolved in sesame oil in such concentration that the daily dose was contained in 0.05 cc. of oil. Excessive luteinization of the ovary was obtained by treatment with pregnant mare serum.

TABLE 1

Survival time of controls, sesame oil treated, and desoxycorticosterone acetate treated adrenalectomized "A" mice

TREATMENT	NUMBER AND SEX OF ANIMALS	AGE	DAILY DOSE	DRINKING WATER	NUMBER OF ANIMALS DYING IN LESS THAN		
					10 days	15 days	15 days
					From beginning of treatment		After cessation of treatment
		days					
Controls	10♀	120		2% saline	10		
	5♂‡	40		2% saline	3	2	
	5♀‡	40		2% saline	4†		
Sesame oil	5♂	45	0.5 cc.	2% saline	4	1	
	5♀	45	0.5 cc.	2% saline	3	1†	
	5♂‡	40	0.5 cc.	2% saline	5		
	5♀‡	40	0.5 cc.	2% saline	5		
Desoxycorticosterone acetate	8♂‡	52	0.25 mgm.	2% saline	7		1
	10♀	180	0.25 mgm.	Tap water			10
	4 C_3H* ♂	90	0.25 mgm.	2% saline	2		2
	5 NH* ♀	150	0.25 mgm.	2% saline	5		
	3 C_3H* ♂	90	0.25 mgm.	Tap water			3
	6 NH* ♀	150	0.25 mgm.	Tap water	1		5

* These two strains of mice were included only to eliminate the possibility that the toxicity of desoxycorticosterone acetate and saline might be strain limited and are, therefore, not listed in "Material and Methods."

† The other animal of this group lived indefinitely due to the presence of accessory adrenal tissue.

‡ Castrated.

The P.M.S.[3] was a water solution and as much as 0.1 cc. was injected in some cases. All hormone injections were started two days before ad-

[2] The estradiol benzoate, testosterone propionate and progesterone were generously supplied by the Schering Corporation through the courtesy of Dr. E. Schwenk. The Ciba Pharmaceutical Products Company, through the courtesy of Dr. E. Oppenheimer, kindly furnished the desoxycorticosterone acetate.

[3] Purified pregnant mare serum "Gonadin" was generously supplied by Cutter Laboratories through the courtesy of Drs. Donald Wonder and C. Parham.

renalectomy and administered daily unless otherwise noted. The diet in all cases consisted of Purina Fox Chow. The drinking water of the adrenalectomized animals contained about 2 per cent sodium chloride except in the few cases listed in table 1.

RESULTS. At the beginning of these experiments untreated adrenalectomized animals survived from 2 to 6 days, but later the minimal survival time increased appreciably. This increase in survival time is ascribed to an improvement in operative technique with consequent diminution of trauma and shock. Throughout the experiment the majority of the animals died before the seventh day. Animals which survived longer than 10 days often lived indefinitely, possibly as a result of the hypertrophy of accessory adrenal tissue. For these animals to survive, it was necessary to continue the saline for 2 or 3 weeks. Desoxycorticosterone acetate was used as a known means of maintaining life after adrenalectomy (table 1). This substance in a daily dose of 0.25 mgm. protected all the adrenalectomized mice which did not receive NaCl in the drinking water. When given saline, however, 7 of 8 animals receiving desoxycorticosterone acetate survived less than 10 days. The saline group chewed at their wounds, often removing a considerable area of skin around the incision. They also showed an increased tendency to eat any animal which died, usually devouring everything but the bones and skin. Edema was observed in those few animals available for autopsy. The effects of desoxycorticosterone acetate and saline were not peculiar to animals of the A strain since similar behavior was noted in mice of the NH and C_3H strains. The fact that some of the C_3H mice survived may have been due to their greater body size since they weighed 28 grams as compared with 20 and 18 grams body weight in the A and NH strains, respectively.

As shown in table 2, progesterone was effective in maintaining life in adrenalectomized mice. Daily injection of 0.5 mgm. maintained life in about half of the animals while 1 mgm. protected completely against the effects of adrenalectomy throughout the period of injection. Upon termination of progesterone injections, all animals died of adrenal insufficiency within the expected time. Saline was given throughout these experiments.

Notwithstanding the protective action of injected progesterone, corpora lutea of pseudopregnancy and of pregnancy were of doubtful effectiveness in this respect. Of 10 mice adrenalectomized 3 to 5 days after copulation with a vasectomized male, only one lived more than 10 days, and this one lived indefinitely, possibly due to hypertrophy of accessory tissue. The corpora lutea of the operated animals showed signs of atrophy, indicating that adrenalectomy affected their normal function. Other female mice adrenalectomized on the twelfth day of pregnancy, or when the placental

sign was first observed, usually died within the range of survival of the untreated, adrenalectomized controls, although 3 animals which either resorbed their fetuses or aborted survived slightly longer.

Female mice which received 20 r.u. of pregnant mare serum daily for 35 days preceding adrenalectomy survived somewhat longer than those re-

TABLE 2

The effectiveness of progesterone and of functional corpora lutea in ameliorating the symptoms of adrenal insufficiency in adrenalectomized "A" mice

TREATMENT	NUMBER AND SEX OF ANIMALS	AGE	DAYS OF PRE-TREATMENT*	TOTAL DAYS TREATED	DAILY DOSE	NUMBER OF ANIMALS DYING IN LESS THAN 10 days (After adrenalectomy)	15 days (After adrenalectomy)	10 days (After cessation of treatment)
		days						
Progesterone	5♂**	65	2	12	1.0 mgm.			5
	5♀**	65	2	12	1.0 mgm.			5
	5♂**	60	2	12	0.5 mgm.	3		2
	5♀**	60	2	12	0.5 mgm.	2		3
Pseudopregnancy	10♀	120				9†		
Pregnancy	10♀	120				7	3	
P.M.S.¶	3♂**	100	65	‡	5 r.u.	3		
	5♂**	100	65	‡	10 r.u.	5		
	10♀	70	35	‡	20 r.u.	4§		
	8♀	70	35	‡	20 r.u.	4§		
	8♀	105	75	‡	20 r.u.	4	4	
	10♂	95	65	‡	20 r.u.	10		

* Number of days before adrenalectomy that hormone treatment was begun.

† The other animal of this group lived indefinitely due to the presence of accessory adrenal tissue.

‡ Treatment was continued until the animals died or it became clear that they would survive indefinitely.

§ The remaining animals in this group survived indefinitely but no evidence of accessory adrenal tissue was visible macroscopically.

¶ The dosage of P.M.S. (pregnant mare serum) is expressed in terms of the Cole-Saunders rat unit which is biologically equivalent to two of the international units recently established.

** Castrated.

ceiving 0.5 mgm. progesterone. The ovaries of these animals showed excessive luteinization and were probably secreting large amounts of progesterone. However, when the P.M.S. was continued for 75 days, the protective action of the ovary became very slight. By this time much of the luteal tissue of the ovary had been replaced by luteal-like cells and apparently progesterone production had fallen below the level needed to protect against adrenalectomy. However, when adrenalectomized animals

were protected by the heavily luteinized ovaries for 10 to 25 days, removal of the ovaries did not prevent their subsequent survival. That this survival was not due to a direct action of the pregnant mare serum is shown by its failure to protect intact males and castrates which had received the same P.M.S. treatment.

The action of the other sex hormones known to be secreted by the stimulated ovary were also tested (table 3). A single dose of 1 γ of estradiol benzoate did not affect the survival time. In daily doses of 1 γ or higher, however, this substance was toxic and caused the death of all the adre-

TABLE 3

Effects of various doses of estradiol benzoate and of testosterone propionate upon the survival time of adrenalectomized "A" mice

TREATMENT	NUMBER AND SEX OF ANIMALS	AGE	DAYS OF PRE-TREATMENT*	TOTAL DAYS TREATED	DAILY DOSE	WEEKLY DOSE	NUMBER OF ANIMALS DYING IN LESS THAN 5 days After adrenalectomy	10 days	20 days
		days							
Estradiol benzoate	5♀	50	0	1	1.0 γ		2	3	
	5♂	35	2	†	1.0 γ		4	1	
	5♀	35	2	†	1.0 γ		5		
	10♀	180	10	1	16.6 γ		3	7	
	10♂	85	35	28		50.0 γ	8	2	
	10♀	85	35	28		50.0 γ	10		
Testosterone propionate	4♂	75	2	†	2.5 mgm.‡			3	1
	4♀	75	2	†	2.5 mgm.‡			2	2

* Number of days before adrenalectomy that hormone injections were begun.

† Injections were continued until the animals died.

‡ A 14 mgm. pellet of testosterone propionate was implanted subcutaneously at the time injections were begun.

nalectomized mice within 5 days. Pretreatment with 16.6 γ estradiol benzoate weekly until the week before adrenalectomy also shortened the survival time. This effect was evidently due to a residue of the injected estrogen in the body at the time of adrenalectomy as shown by the non-effect of a single injection of estrogen given 10 to 30 days before adrenalectomy. Both immature and mature females were used in the latter experiment to control any possible effect of the corpora lutea which form in estrus, but not in immature, female mice following a single injection of estrogen. These corpora lutea, like those of pseudopregnancy, were ineffective. Testosterone propionate did not shorten the survival time

even when injected in daily doses of 2.5 mgm. in addition to a 14 mgm. pellet implanted subsequent to the adrenalectomy. All the pellets became infected in the adrenalectomized mice, although such an occurrence was not observed in intact animals. The survival time of the mice treated with testosterone propionate was possibly slightly increased but probably not sufficiently to be significant. There was no evidence of any sex difference in response to any of the hormones tested.

Discussion. It is evident from the above results that the production of progesterone and male hormone by the mouse ovary can be distinguished by adrenalectomy. The observation that progesterone in sufficient quantity will protect against the fatality of adrenalectomy in mice is in agreement with the results which have been obtained with other species studied (4, 5, 6, 7, 9, 10). However, the fact that between 0.5 mgm. and 1.0 mgm. of progesterone is required for this protection makes the mouse agree more nearly with the rat (4, 5, 6, 7) than with the ferret (10) or cat (9) if the differences in body weights are taken into account. The protection appears to be of a different character from that provided by desoxycorticosterone acetate in that the progesterone does not markedly affect the salt and water balance (4), while desoxycorticosterone acetate, if administered in appreciable quantities (0.25 mgm. daily) in conjunction with increased salt intake, causes sufficient salt and water retention to be fatal. However, mice treated with desoxycorticosterone acetate survive readily on a normal diet and salt intake (13). Similar observations have been recorded for man (14).

The fact that 35 days' pretreatment with P.M.S. protects against adrenalectomy, while pretreatment for 75 days gives only a slight increase in survival time over that of the controls, indicates that up to 35 days appreciable quantities of progesterone are being produced by the heavily luteinized ovaries, whereas by 75 days progesterone production has diminished below the level required for maintenance of life in the adrenalectomized mouse. This is apparently associated with the replacement of the true luteal tissue by luteal-like cells and the production of appreciable quantities of male hormone (12). It is of interest, however, that adrenalectomized animals which have been maintained for some time by the luteinized ovaries may be castrated and still survive. Since no accessory tissue was discovered, although all possibilities of its presence have not been exhausted, it may be that the survival is due to an adjustment of the organism to the lack of adrenal cortical hormone (15, 16) which takes place during the period that the animal is maintained by the luteinized ovary.

The mouse seems to differ from some of the other species (8, 10, 17, 18) in that pseudopregnancy and pregnancy do not protect against the consequences of adrenalectomy. This absence of protection indicates that

even though the ovary can be stimulated to produce adequate amounts of progesterone to protect against adrenal insufficiency, it either does not do so under normal conditions or the period of great activity of the corpora lutea is too short to be effective.

In agreement with the findings on other animals thus far tested (2, 19, 20, 21), estrogen in appreciable amounts is very toxic in adrenalectomized mice. In a minimal estrus-producing dose, however, estrogen has neither harmful nor beneficial effects. This observation is in agreement with certain results which have been obtained with rats (22). Male hormone has been reported to be both toxic (4) and noneffective in adrenalectomized rats (11). However, in the present experiment with mice, testosterone propionate, even in the large doses employed (2.5 mgm. daily plus a 14 mgm. pellet), was probably not beneficial, but was clearly not harmful.

No significant difference was observed between oil-treated and uninjected adrenalectomized mice even though sesame oil has been reported to contain a principle which prolongs life in adrenalectomized pregnant rats (23).

SUMMARY AND CONCLUSIONS

Adrenalectomized normal and castrated mice of the A strain survive 3 to 7 days. Administration of testosterone propionate is not harmful to adrenalectomized mice and is probably not helpful, while estradiol benzoate in any but the most minimal dose is toxic. Progesterone in 1 mgm. daily doses maintained life in all of 10 adrenalectomized mice treated; 5 of 10 animals survived on 0.5 mgm. daily. Desoxycorticosterone acetate was effective in ameliorating the symptoms of adrenal insufficiency, but was very toxic even in doses of 0.25 mgm. if given in conjunction with 2 per cent salt in the drinking water. Pregnant mare serum was ineffective in males and castrates, but pretreatment of females for 35 days before adrenalectomy luteinized the ovaries to such an extent that the survival period fell between that following 0.5 mgm. and 1.0 mgm. doses of progesterone. Longer periods of pretreatment (75 days) were less effective. Pregnancy and pseudopregnancy had little if any protective action. It can be concluded, therefore, that progesterone in sufficient amounts, whether produced by the ovary or injected, protects against the fatality of adrenalectomy in mice and that adrenalectomy may be used to distinguish between the endogenous production of large amounts of progesterone and male hormone.

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THE EFFECT OF ETHER ANESTHESIA UPON CERTAIN BLOOD ELECTROLYTES[1]

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The well-known decrease in blood CO_2 capacity produced by ether anesthesia has been studied by many investigators. Henderson and his co-workers (1, 2) have supposed that the respiratory excitement induced by ether caused an alkalosis by the blowing off of CO_2. It is suggested that under these conditions alkali passes into the tissues. Other investigators (3, 4, 5, 6) have reached a different conclusion. They find that the decrease in CO_2 capacity is accompanied by an equally rapid fall in plasma pH and by increase in CO_2 tension. Coincident with these events lactic acid appears in the blood in amounts sufficient to account in large part for the acidosis (6). More recently, Henderson (7) has restated his original contention.

In view of these facts it has seemed desirable to investigate the major electrolyte changes which occur during ether anesthesia.

METHODS. Dogs which had received water but no food since the previous day were placed on the animal board for two hours before the experiments were started. Ether was administered by the drop method and anesthesia was maintained for approximately one hour at such a depth that the corneal reflex was just absent. Complete narcosis with a minimum of struggling was attained within two minutes. Blood samples were drawn without stasis from the jugular veins.

The CO_2 capacity. The CO_2 content of oxalate-fluoride treated whole blood equilibrated at 38°C and 40 mm. Hg pressure CO_2 was determined by the method of Van Slyke and Neill (8).

Lactate. Whole blood was treated with oxalate and fluoride in the usual manner. Lactic acid was measured by Friedemann, Cotonio and Shaffer's (9) method.

Sodium and chloride. Plasma sodium was analyzed according to Butler and Tuthill (10) and plasma chloride was determined by Eisenman's technic (11).

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RESULTS. In seven experiments on five dogs, ether anesthesia for one hour caused an average decrease of 5.4 mM. in the CO_2 capacity. Table 1 shows that there was considerable variation in the bicarbonate decrease

TABLE 1

Effect of ether anesthesia on the CO_2 capacity and the lactic acid content of whole blood

(CO_2 capacity of dog's whole blood measured at 38.1° ± 0.1°C., pCO_2 = 40 mm. Hg; H_2CO_3 = 1.1 mM/L.)

All figures in mM/liter

DOG NUMBER	PERIOD OF ANESTHESIA			RECOVERY PERIOD		
	Duration of anesthesia	$BHCO_3$	Lactic acid	Duration of recovery	$BHCO_3$	Lactic acid
	min.			*min.*		
1	0	19.7				
	7	15.2				
	23	14.9				
	43	13.6				
2	0	19.0				
	15	14.7				
	32	15.6				
	62	13.5				
2	0	17.4	1.9	199	16.9	2.0
	11	14.4	3.6			
	59	10.8	6.4			
3	0	17.4				
	22	13.7				
	45	13.0				
	59	14.0				
3	0	15.9	0.6	60	13.4	5.2
	10	15.5	1.9			
	60	11.1	6.8			
4	0	14.9	1.8	118	14.0	2.1
	10	7.9	6.9			
	57	8.8	6.9			
5	0	12.5	1.3	36	12.5	3.8
	10	10.0	7.4			
	60	7.1	7.1			

which ranged between 3.4 and 6.6 mM. Even in experiments carried out upon the same animal (2 and 3) at different times the agreement was only qualitative. It is of particular interest to note that in general the CO_2

capacity decreased progressively with the duration of anesthesia. In those experiments in which this was not the case the aberrant points may be ascribed to a decrease in the depth of narcosis. Approximately two-thirds of the fall in the CO_2 capacity which was present at the end of one hour of anesthesia occurred during the first ten minutes.

The effect of ether anesthesia upon the blood lactic acid concentration was determined in four experiments (table 1). At the end of one hour the average increase in lactic acid was 5.4 mM. This value ranged in different experiments between 4.5 and 6.2 mM.

While the increase in blood lactate concentration may be larger or smaller than the decrease in CO_2 capacity in any given blood sample, the average increase in blood lactate after 10 minutes' etherization was 3.5 mM. in four experiments, which agrees well with an average decrease in bicarbonate of 3.2 mM. At the end of one hour these values were 5.4

TABLE 2

The relation between the diminished alkaline reserve and increased concentration of lactic acid during ether anesthesia

DOG NUMBER	AT 10 MINUTES		AT 1 HOUR	
	$BHCO_3$ decrease	Lactic acid increase	$BHCO_3$ decrease	Lactic acid increase
	mM/liter	*mM/liter*	*mM/liter*	*mM/liter*
3	0.4	1.3	4.8	6.2
2	3.0	1.7	6.6	4.5
4	7.0	5.1	6.1	5.1
5	2.5	6.1	5.4	5.8
Average......	3.2	3.5	5.7	5.4

mM. for lactic acid and 5.7 mM. for bicarbonate (table 2). Although it is recognized that other changes in the acid-base balance have occurred, these results indicate that the increase in lactate is of the proper order of magnitude to account for the observed fall in CO_2 capacity.

Analyses made at varying periods after the anesthesia (table 1) showed that the excess lactate may be removed within one and a half hours. There was a simultaneous increase in the bicarbonate concentration.

In eight experiments plasma sodium and chloride analyses were made before, at the end of one hour of anesthesia, and approximately one hour after the withdrawal of ether (table 3). There was no significant change in the concentration of either electrolyte.

Since the withdrawal of appreciable amounts of blood was necessary for the various analytical procedures employed, hemorrhage might be assumed to be a factor in the results obtained. This point is particularly pertinent for it is known that the excessive loss of blood decreases the

alkali reserve and increases the blood lactate (12, 13 and others). Two control experiments in which every step except etherization was repeated are shown in table 4. Since the concentrations of bicarbonate and lactic acid are relatively unchanged, it can be assumed that the above data are not complicated by the effects of hemorrhage.

TABLE 3

Electrolyte analyses before, during and after one hour of ether anesthesia

DOG NO.	Na^+ IN MEQ. PER LITER OF SERUM					Cl^- IN MEQ. PER LITER OF SERUM				
	Control	Ether	Difference	Recovery	Difference	Control	Ether	Difference	Recovery	Difference
6	153.2	157.0	+3.8	152.4	−0.8	110.2	109.0	−1.2	108.4	−1.8
7	148.5	149.6	+1.1	146.7	−1.8	111.0	110.0	−1.0	113.2	+2.2
8	150.4	148.6	−1.8	149.0	−1.4	108.6	106.8	−1.8	106.9	−1.7
3	143.9	142.0	−1.9	141.0	−2.9	117.8	119.4	+1.6	118.1	+0.3
2	146.1	149.5	+3.5	145.3	+0.8	118.1	120.3	+2.2	120.2	+2.1
9	144.1	145.6	+1.5	146.3	+2.2	117.8	118.4	+0.6	118.9	+1.1
5	150.3	150.8	+0.5	149.8	−0.5	120.0	121.0	+1.0	119.0	−1.0
4	149.0	149.5	+0.5	149.5	+0.5	119.5	120.0	+0.5	121.0	+1.5
Average..			+0.9		−0.5			+0.2		+0.3

TABLE 4

Control experiments—no anesthesia

(CO_2 capacity of dog's whole blood measured at 38.1 ± 0.1°C., pCO_2 = 40 mm. Hg; H_2CO_3 = 1.1 mM/L.)

DOG	WEIGHT	TIME	$BHCO_3$	LACTIC ACID	BLOOD DRAWN
	kgm.	*min.*	*mM/L.*	*mM/L.*	*cc.*
10	20.0	0	17.2	2.2	20
		29	18.0	2.8	16
		94	18.2	3.0	20
		192	17.7	3.0	16
					72
11	13.1	0	14.8	1.6	25
		29	15.6	2.3	15
		98	15.0	2.8	17
		197	16.0	2.2	20
					77

DISCUSSION. According to Lipow, Weaver and Reed (14) the administration of ether to dogs generally decreased the blood sodium concentration during the first hour. Following this there was a pronounced increase. Fay, Andersch and Kenyon (15) found that one hour of ether anesthesia

one hour of ether administration can be accounted for largely by the increase in blood lactate concentration (4.5 to 6.2 mM.).

4. After ether anesthesia the return of the lactate and the CO_2 capacity to the control values occurs in about one and one-half hours.

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CALCIUM IN THE COAGULATION OF THE BLOOD[1]

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It is generally accepted that thrombin is formed from the interaction of prothrombin, thromboplastin and calcium, but much uncertainty remains concerning the manner in which calcium functions in this reaction. In the present paper the rôle of this element is studied under conditions in which the other two components of the reaction are made constant. It has been shown by the author (1) that the prothrombin is relatively constant for any one species. Thromboplastin can easily be made a constant by adding an excess of the reagent. For this study chicken blood is well adapted since it can be maintained fluid without the addition of any anticoagulant, thereby avoiding the introduction of outside factors other than thromboplastin itself.

Blood was drawn from the wing vein into a syringe which had been chilled with ice, and transferred into collodion-coated test tubes which were immersed in an ice bath. Doctor Hirschboeck (2) has recently discovered that collodion is more efficient in retarding coagulation than paraffin and this observation has been repeatedly verified in the writer's laboratory. The plasma was obtained by centrifuging the blood in an angle centrifuge at 3,000 r.p.m. This plasma at the temperature of the refrigerator remained fluid for several weeks. For the preparation of thromboplastin 0.1 gram of chicken brain (dehydrated with acetone according to the author's method for rabbit brain (3)) was mixed with 5 cc. of 0.85 per cent sodium chloride solution and incubated for 20 minutes at 50°C.

To determine quantitatively the effect of thromboplastin on the clotting time, 0.1 cc. of plasma was mixed in a small test tube with 0.2 cc. of saline solution containing varying concentrations of the thromboplastin emulsion. The tube was put into a water bath kept at $37\frac{1}{2}$°C., and the clotting time recorded with a stop watch. The results are presented in figure 1. It will be noted that as the amount of thromboplastin was progressively increased, the clotting time rapidly decreased until a certain concentration was reached, after which the rate remained constant irrespective of the excess

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of thromboplastin. This finding is exceedingly important since it enables one to make the thromboplastin factor a constant merely by adding enough of the agent to obtain the minimal optimum concentration. The experiment presents strong evidence that the absence or delay in the coagulation of chicken blood is probably due solely to a lack of thromboplastin, and it furthermore demonstrates that an exceedingly small amount of this agent is sufficient to bring about coagulation in the time which is considered within the normal range.

It is fairly certain that circulating blood does not contain free or available thromboplastin. This agent is apparently liberated only after the blood is shed; and the rate at which it becomes available determines the

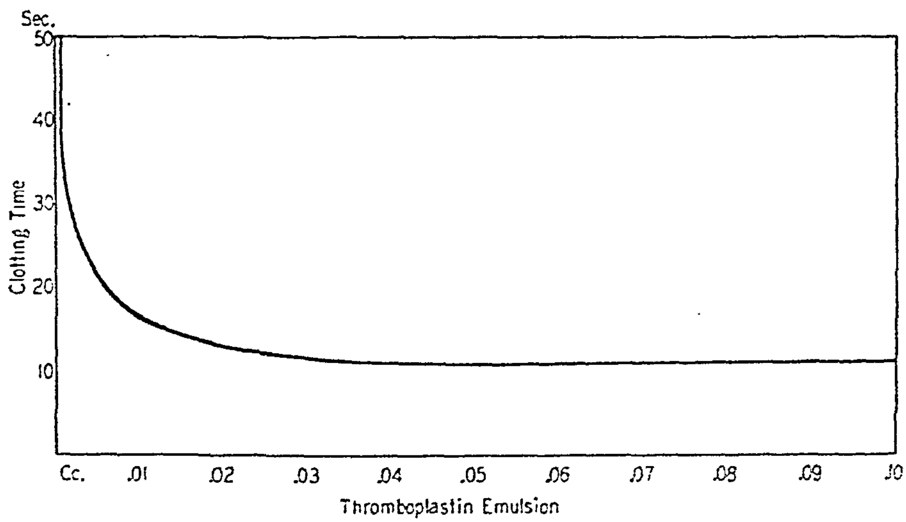

Fig. 1. The relationship of the clotting time to the concentration of thromboplastin. For the determination, 0.1 cc. of chicken plasma (containing no decalcifying agent) was mixed with 0.2 cc. of physiological saline solution containing varying amounts of thromboplastin emulsion.

coagulation time. Three important factors must be considered in the liberation of thromboplastin: 1, species; 2, temperature; and 3, surface of container. In regard to species it is well known that goose blood, if not contaminated with thromboplastin from tissue juices, will remain fluid in an ordinary glass container. Likewise horse blood will not clot in a glass receptacle provided it is kept in an ice bath. Human and rabbit bloods on the contrary coagulate rapidly even when cooled in ice, but the low temperature nevertheless has a demonstrable retarding effect on the clotting time of these bloods. The third factor, the surface of the container, is of utmost importance. Freund (4) as early as 1886 recorded that a coating of vaseline retarded coagulation, and later Bordet and Gengou (5) demonstrated that paraffin was better and more convenient. It has now been found that collodion is even superior to paraffin (2). The comparison

of the effect of glass and collodion on coagulation is shown in table 1. From these results it can be seen that coagulation is greatly retarded in a collodion-coated tube and that less oxalate is required to maintain the fluidity of human and rabbit blood. These differences in clotting are due without doubt to variations in the liberation of thromboplastin. Ferguson (6) as well as earlier investigators have shown that calcium is an important factor in the lysis of platelets, and thus it seems logical to assume that even partial removal of the calcium of the blood will decrease the rate of platelet destruction with the result that less thromboplastin is liberated. Excess

TABLE 1

The influence of the surface of the container on the amount of sodium oxalate required to inhibit coagulation

SPECIES	CONTAINER	SODIUM OXALATE 0.1 M ADDED TO 5 CC. OF BLOOD						
		0	0.05 cc. (0.001 M)*	0.075 cc. (0.0015 M)	0.10 cc. (0.004 M)	0.15 cc. (0.003 M)	0.20 cc. (0.004 M)	0.25 cc. (0.005 M)
Goose	Glass	No clot						
	Collodion	No clot						
Chicken	Glass	Clot	Clot	Clot	Clot	No clot		
	Collodion	No clot						
Man	Glass	Clot	Clot	Clot	Clot	Clot	No clot	
	Collodion	Clot	Clot	No clot				
Rabbit	Glass	Clot	Clot	Clot	Clot	Clot	Clot	No clot
	Collodion	Clot	Clot	Clot	Clot	Clot†	No clot	

The blood was drawn into a cold syringe, mixed immediately with sodium oxalate, centrifuged, and then placed in a refrigerator.

* The figures in parentheses indicate the calculated molar concentration obtained by diluting the sodium oxalate to 5 cc.

† A few threads of fibrin appeared after several hours.

oxalate will in addition remove the calcium needed for converting prothrombin to thrombin, but it is obvious that the clotting time of blood furnishes no significant information concerning the fraction of calcium which actually takes part in the coagulation reaction.

The calcium required for converting prothrombin to thrombin. In order to study successfully the calcium factor in coagulation, it is necessary to make the other constituents of the reaction, namely, prothrombin and thromboplastin, constants. Since the concentration of prothrombin for any one species is fixed (1), only thromboplastin needs to be considered. It has already been shown, in figure 1, that this can easily be done by adding an excess of this reagent. Therefore the problem actually resolves

itself into determining how much calcium must be removed before coagulation is inhibited in the presence of an optimal concentration of thromboplastin. This is readily accomplished by adding varying amounts of sodium oxalate to a fixed amount of blood, and then determining the coagulation time of the plasma after an excess of thromboplastin has been added. The results of this experiment on chicken and human bloods are presented in table 2.

Several conclusions can be drawn from these results. It is clear that if just sufficient sodium oxalate is added to precipitate the total calcium of the blood, coagulation is not inhibited. To prevent clotting 0.2 cc. of

TABLE 2

The quantity of sodium oxalate required to inhibit coagulation in the presence of excess thromboplastin

	Sodium oxalate 0.1 M added to 5 cc. of blood						Species
	0	0.05 cc. (0.001 M)*	0.075 cc. (0.0015 M)	0.10 cc. (0.002 M)	0.20 cc. (0.004 M)	0.30 cc. (0.006 M)	
	Clotting time in seconds						
2 hours after adding sodium oxalate	11	11	13	22	180	No clot	Chicken
10 min. after adding sodium oxalate	11	11	16	45	No clot	No clot	Man
1 hour after adding sodium oxalate		11½	45	†	No clot	No clot	Man

The clotting time was determined by mixing 0.1 cc. of the oxalated plasma with 0.1 cc. of physiological saline solution, and 0.1 cc. of thromboplastin emulsion.

* The figures in parenthesis indicate the calculated molar concentration resulting from diluting the sodium oxalate to 5 cc. Blood containing 6 mgm. of calcium per 100 cc. of whole blood has a 0.0015 M concentration of calcium. Therefore the addition of 0.075 cc. of 0.1 M sodium oxalate to 5 cc. of blood supplies the molar equivalency of the calcium content.

† A few shreds of fibrin formed after one hour.

0.1 M sodium oxalate must be added to 5 cc. of blood. At this dilution the sodium oxalate is 0.004 M. Since the concentration of calcium is approximately 0.0015 M (6 mgm. per 100 cc. of blood), it can be concluded that somewhat less than 3 times the amount of sodium oxalate required to precipitate the total calcium is necessary to prevent coagulation in the presence of an optimal concentration of thromboplastin.

It is to be further noted that the action of sodium oxalate is not instantaneous. To study this time factor more accurately, whole blood is not particularly suitable. Better adapted for this study is plasma containing no anticoagulant agent, but maintained fluid by keeping it in a collodion-coated test tube cooled by means of ice. The experiment was

performed by mixing 0.5 cc. of plasma with varying amounts of sodium oxalate, and after definite time intervals, taking 0.1 cc. of the oxalated plasma, mixing it with 0.1 cc. of saline and 0.1 cc. of thromboplastin emulsion and then determining the clotting time. The results which were obtained are summarized in table 3. The important fact brought out by this study is that the anticlotting action of sodium oxalate is not immediate, but requires a measurable period of time. The higher its concentration, the shorter the period required to bring about complete inhibition of coagulation. Again it is found that an amount of oxalate equivalent to approximately 3 times the total calcium of the plasma (10 mgm. per 100 cc. of plasma) will, if allowed to react for several minutes before

TABLE 3

The time factor in the inhibition of coagulation by sodium oxalate

TIME OF INCUBATION	SODIUM OXALATE 0.1 M ADDED TO 0.5 CC. OF PLASMA (CONTAINING NO DECALCIFYING AGENT)						
	0	0.01 cc. (0.002 M)	0.02 cc. (0.004 M)	0.03 cc. (0.006 M)	0.04 cc. (0.008 M)	0.05 cc. (0.010 M)	
	Clotting time in seconds						
10 sec.	11	11½	12	16	17	20	Chicken plasma
1 min.		12	14	75	300	No clot	
5 min.		12½	35	360	No clot	No clot	
30 min.		12½	140	660	No clot	No clot	
10 sec.	11	11	12	12	14	20	Human plasma
1 min.		10½	17	165	300	No clot	
5 min.		*	*	No clot	No clot	No clot	

The clotting time was determined by mixing 0.1 cc. of the oxalated plasma with 0.1 cc. of saline solution and 0.1 cc. of thromboplastin emulsion.

* Plasma clotted spontaneously.

thromboplastin is added, prevent completely coagulation though an excess of the latter factor is present. Interestingly, when 0.02 cc. of 0.1 M sodium oxalate is added to 0.5 cc. of chicken plasma, the retarding action on coagulation is slow but definitely progressive, and at the end of 30 minutes the clotting time is increased from 11 to 140 seconds. The same amount of oxalate added to human blood likewise begins to inhibit coagulation, but before 5 minutes elapse, enough thromboplastin is liberated, presumably from the platelets, to cause spontaneous coagulation. It is easy to see that an amount of sodium oxalate sufficient to inhibit ultimately coagulation, is inadequate if during this period thromboplastin is liberated. Obviously consistent results cannot be obtained unless the thromboplastin factor is controlled, and in the light of this it is easy to understand why conflicting findings were frequently obtained in the past.

Certain practical considerations arise from the present work. It is often observed that blood containing an adequate amount of oxalate or citrate will, nevertheless, on standing contain a film or veil of fibrin. If such blood be used for transfusions, the danger of emboli is encountered. No satisfactory explanation for this slight coagulation has been given, but on the basis of the present findings it seems reasonable to suppose that the fundamental cause is the slow decalcifying action of oxalates and citrates. During the time the active calcium is removed, sufficient thromboplastin may become liberated to convert a small amount of prothrombin to thrombin and although only a minute quantity of the latter may be formed, it is sufficient to clot enough fibrinogen to form a visible film of fibrin. To prevent this incipient coagulation, it is not only necessary to employ a definite excess of sodium citrate or oxalate, but also to prevent the liberation of thromboplastin. Among the means to accomplish the latter are: chilling the blood; using chemically clean containers (perhaps substituting a plastic such as Lampert's athrombit (7) for glassware); and avoiding air bubbles and foaming. It appears certain that the interface, air-blood, is an underestimated factor in liberating thromboplastin.

Certain conclusions concerning the action of calcium can be drawn from the present results. It appears certain that ionized or free calcium does not take part in the conversion of prothrombin to thrombin. If it were the unbound calcium, the anticlotting action of sodium oxalate would be immediate since the removal of the ionized calcium would not require the time interval observed in the experiments of table 3. The most logical interpretation of the findings recorded in this study is that prothrombin itself contains calcium, and that like proteins is weakly dissociated or ionized. In blood one finds the equilibrium:

$$\text{Prothrombin Ca} \rightleftharpoons \text{Prothrombin}^{=} + \text{Ca}^{++}$$

It is easy to understand why an equivalent amount of sodium oxalate does not inhibit coagulation for the insoluble calcium oxalate likewise dissociates:

$$\text{Ca oxalate} \rightleftharpoons \text{Ca}^{++} + \text{oxalate}^{=}$$

Consequently as long as there is a sufficient concentration of calcium ions, the prothrombin complex remains intact. From the law of mass action one knows that:

$$\frac{\text{Ca}^{++} \times \text{oxalate}^{=}}{\text{Ca oxalate}} = K_1$$

and

$$\frac{\text{Ca}^{++} \times \text{prothrombin}^{=}}{\text{Prothrombin Ca}} = K_2$$

An equilibrium is thus established between the two systems. From table 3 one can deduce that if slightly less than 0.02 cc. of 0.1 M sodium oxalate is added to 0.5 cc. of chicken plasma enough calcium ions remain in solution to preserve the prothrombin Ca complex. If, however, more oxalate is added to plasma, the calcium ions are depressed and at a certain concentration the calcium is completely torn from the prothrombin molecule. At this point, the prothrombin is no longer able to react with thromboplastin and therefore coagulation is completely inhibited. According to this concept prothrombin in its normal state as it occurs in the blood is a calcium compound which is converted to thrombin as soon as it reacts with thromboplastin.

In oxalated plasma, no true or normal prothrombin occurs but only an inactive decalcified derivative. On recalcifying oxalated plasma, prothrombin is quickly regenerated according to the following equation:

$$\underset{\text{(Inactive form)}}{\text{Prothrombin}} + \text{Ca} \rightarrow \underset{\text{(True active form)}}{\text{Prothrombin Ca}}$$

In contrast to decalcification which is a relatively slow process, recalcification is practically instantaneous indicating that prothrombin has a great avidity for calcium. This is strikingly illustrated by a rather simple series of experiments. By mixing plasma containing a fixed excess of oxalate with thromboplastin and then adding calcium chloride solution, optimal coagulation was obtained for a wide range of calcium concentrations as shown in table 4. Theoretically, a calcium chloride concentration of 0.0075 M will precipitate all of the oxalate contained in the plasma, but curiously a concentration as low as 0.00065 M is still able to cause clotting. From 0.0025 to 0.025 M the clotting time is fairly constant and corresponds rather closely to the rate observed after adding thromboplastin to plasma containing no anticoagulants. This suggests that for these concentrations of calcium, the prothrombin is quickly and completely regenerated and will cause clotting in the period normally observed for a fixed amount of prothrombin. Naturally, for low or inadequate amounts of calcium not all of the prothrombin can be reformed. For higher levels of calcium the depressing action of this ion (8) begins to manifest itself.

If calcium chloride is added first to the oxalated plasma, followed by thromboplastin one minute later, strikingly different results are obtained. Weak solutions of calcium, i.e., below 0.0025 M, no longer are sufficient to cause clotting, and even higher concentrations are inadequate to bring about clotting within the normal time of 11 seconds. But a definite excess of calcium such as is obtained by adding a 0.02 M solution will cause clotting in about 7 seconds. This can be easily explained. By incubating oxalated plasma with excess calcium chloride, the thromboplastin of the plasma has a chance to react with the prothrombin before the

thromboplastin emulsion is added, and therefore some thrombin is already present before the main reaction occurs with the result that the clotting time is definitely shortened. By mixing oxalated plasma with an inadequate amount of calcium chloride before adding thromboplastin, a distribution of the calcium can take place with the establishment of an equilibrium of the calcium held by the oxalate, the proteins and the prothrombin. Thus the amount of prothrombin Ca which exists for any particular equilibrium determines the coagulation time.

One important practical deduction to be made from this study is that the method of determining quantitatively prothrombin by the clotting time

TABLE 4

Effect of the concentration of calcium chloride on the clotting time when added to oxalated plasma containing an excess of thromboplastin and when allowed to react with oxalated plasma before thromboplastin is added

MOLAR CONCENTRATION OF CALCIUM CHLORIDE	CLOTTING TIME IN SECONDS	
	Plasma mixed with thromboplastin before calcium chloride was added	Thromboplastin added 1 minute after plasma was mixed with calcium chloride
0.250	11½ to 12	7 to 8
0.200	11 to 12	7 to 8
0.015	11 to 12	11 to 12
0.010	11 to 11½	13 to 23
0.005	10 to 11	60 to 200
0.0025	10 to 11	300 to 500
0.00125	13 to 17	No clot
0.00062	60 to 90	No clot

For the determination 0.1 cc. of plasma, 0.1 cc. of thromboplastin, and 0.1 cc. of calcium chloride were employed.

One cubic centimeter of 0.1 M sodium oxalate was added to 9 cc. of blood. On the basis that whole blood contains 6 mgm. per 100 cc., the sodium oxalate concentration of the plasma in excess of the calcium is approximately 0.0075 M.

of recalcified plasma containing an excess of thromboplastin has for its support the significant observation that the clotting time of normal plasma to which an excess of thromboplastin is added is essentially the same as that of oxalated plasma which is mixed with thromboplastin and a fixed quantity of calcium chloride. This observation has not only been made on chicken and human bloods, but also on the bloods of the horse, rabbit, dog, and other animals.

The conclusion that it is the combined calcium which is responsible for the conversion of prothrombin to thrombin is not new. The fact that to prevent clotting 3 times more oxalate must be added than is required to precipitate the total calcium as Vines, (9) Scott and Chamberlain (10) have found has led Vines and Collingwood (11) to conclude that the free or

ionized calcium does not enter into the coagulation mechanism. Recently Ferguson (12) obtained evidence that a calcium containing intermediary complex is formed during the conversion of prothrombin to thrombin. It is difficult to see from his data how one could differentiate between normal prothrombin which according to these present studies is a calcium compound, and this intermediary complex. From the data present, one must conclude that the calcium is already bound to the prothrombin before it reacts with thromboplastin. After the completion of this paper, Martin (13) published the results of a study on the action of various decarboxylic acids on the clotting time. He likewise reached the conclusion that prothrombin is a calcium compound and he postulates that prothrombin in its conversion to thrombin is first decalcified and then acted upon by ionized calcium. This differs from the writer's conclusion that prothrombin is directly converted to thrombin by means of thromboplastin without ionized calcium.

SUMMARY

1. Addition of increasing quantities of thromboplastin to native chicken plasma (not decalcified) causes a progressive increase in the speed of coagulation until a fixed minimal time is reached after which the rate remains constant irrespective of the excess of thromboplastin.

2. The speed with which blood (uncontaminated with tissue juice) coagulates is determined by the rate with which thromboplastin is liberated. Among the important factors influencing the latter are: 1, species; 2, temperature; and 3, surface of container. The more the liberation of thromboplastin is inhibited, the less the amounts of decalcifying agents such as oxalates and citrates needed to prevent coagulation.

3. To determine the absolute amount of sodium oxalate required to inhibit coagulation, specimens of blood containing increasing known amounts of oxalate are tested by mixing a definite volume of the plasma with a fixed excess of thromboplastin. Approximately 3 times the amount of oxalate calculated to precipitate the total calcium is required to prevent the clotting of chicken and human bloods.

4. The anticoagulating action of sodium oxalate is not immediate. The greater the excess of oxalate the faster the coagulation is inhibited. By testing plasma with excess thromboplastin after definite time intervals following the addition of oxalate, no inhibition is found if slightly less oxalate is added than the amount calculated to precipitate all the calcium. In the case of human blood an amount of oxalate approximately $2\frac{1}{2}$ times the calculated calcium equivalent of the plasma will inhibit clotting completely within 5 minutes whereas a 4-fold excess stops clotting in less than 1 minute.

5. Oxalated plasma containing excess thromboplastin will on recal-

cification clot promptly, and for a wide range of calcium chloride concentrations coagulation will occur in approximately the same time as is observed for unoxalated plasma mixed with excess thromboplastin.

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THE EFFECT OF ADRENALECTOMY AND OF FASTING ON THE FUNCTIONAL CAPACITY OF THE RAT'S GASTROCNEMIUS

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Although muscular weakness has long been observed to accompany corticoadrenal insufficiency, it is not clear to what extent such incapacity of the muscle may be due to a deficiency within the muscle itself, and to what extent nervous and circulatory factors may be responsible for the apparent muscular inadequacies. The observations of Hartman and Lockwood (1) on the fatigability of the synapses and myoneural junction in adrenalectomized rats seem to indicate that the nervous system is affected to a sufficiently profound degree to account for much if not all of the observed asthenia of adrenal insufficiency. Ingle (2–5) has demonstrated that muscles of adrenalectomized animals, when directly stimulated three times a second, cease to respond sooner than do those of normal animals, and do less work during the period that they do respond. Under the conditions of his experiments, however, a normal muscle continues to contract for many days, and in the case of an adrenalectomized animal the muscle stops contracting only when the whole animal collapses. The length of time during which the muscle continues to contract under these conditions, as well as the amount of work which it does, does not necessarily bear a relationship to the condition of the muscle at the beginning of the experiment, but may be merely a reflection of the ability of the circulatory system to maintain adequate conditions for the recovery of the muscle between contractions. Circulatory disturbances, possibly profound enough to account for his results, are a well known feature of adrenal insufficiency. We have therefore reinvestigated the effect of adrenal insufficiency on muscle function by causing the muscle to contract maximally and continuing the stimulation at such a rate that the muscle would remain in complete tetanus until fatigued. Since under these conditions the muscle has little time for recovery, it is believed that the total performance will more nearly reflect the condition of the muscle itself.

METHOD. Female rats of seven to eight weeks of age were used. Adrenalectomy was performed by the usual dorso-lateral approach at one stage. Muscle tension experiments were performed when the adrenal-

ectomized animals showed signs of adrenal insufficiency as judged by weight loss. The controls were matched for age and weight with the experimental animals, and were from the same litters whenever practicable. They were normal in every particular except that a sham adrenalectomy was performed; to control the factor of inanition which accompanies adrenal insufficiency, another group had no operation, but were fasted to the same weight loss as occurred in the adrenalectomized rats.

In a tension experiment, the animal was anesthetized with ether, the femur fixed vertically in a clamp, and the tendon of the left gastrocnemius attached to a Blix type of torsion rod of medium resistance, with optical recording which gave a magnification of 322 to 1. Maximal stimuli were delivered from the secondary of an inductorium at the rate of 60 a second through electrodes in contact with the muscle. Stimuli were continued and the isometric response continuously recorded until it was judged that the muscle was no longer exerting tension. The gastrocnemius was then dissected out and weighed.

Observations. Forty-eight animals were used in these experiments, 16 in each group. The maximum tension was developed early within the first second of stimulation, and usually the tension began to decline almost immediately, rather rapidly during the first twenty-five seconds, and then more and more slowly, so that near the end of the record it was very difficult to judge with certainty exactly when the base line was reached. For this reason we have arbitrarily considered the muscle "fatigued" when the tension declined to 10 per cent of the maximum. The "fatigue time," then, is the time from the beginning of stimulation to the point where the muscle is exerting only 10 per cent of its original maximum tension, keeping the rate and intensity of the stimulation constant throughout.

The maximum tension exerted by the sham-operated rats averaged 1977 grams per gram of muscle; the average for the fasted rats was 2084 grams per gram, and for the adrenalectomized rats, 1862 grams per gram. Since the standard errors $\left(\frac{\sigma}{\sqrt{n}}\right)$ for these three averages were 66, 84 and 72, respectively, it is evident that there was no significant difference in the absolute strengths of the muscles of the various groups of animals. The only other measurement of the absolute strength of muscles from adrenalectomized animals of which we are aware was made by Gans and Miley (6), by a method of trial and error, hanging weights on a Harvard type muscle lever, and determining how great a weight the muscle would lift when stimulated by single shocks. They also concluded that adrenalectomy did not reduce the absolute strength of the rat's gastrocnemius.

The average fatigue time for each group of muscles was as follows: sham-operated rats, 46.2 seconds; fasted, 36.1 seconds; adrenalectomized, 32.5 seconds. The differences between these means seem to be of a

considerable order of magnitude, but they are of doubtful significance, since the standard errors of the means are 4.1, 2.1 and 3.0, respectively.

Although the maximum strength of the muscles from adrenalectomized rats was as great as that of the muscles from the other groups, and the fatigue time was only slightly shorter, nevertheless their performance was distinctly inferior. This fact is evident from an inspection of table 1, which shows that during the early course of the stimulation the tension declines more rapidly in the adrenalectomized group than in the others, so that by the end of the fifth second it is significantly lower, and becomes increasingly so until about the fifteenth second. From that point on, the three curves run approximately parallel, although the tension exerted by the adrenalectomized group remains significantly lower than the others. The decline in tension in the fasted group roughly parallels that of the sham-operated group throughout, so that at no time during the stimulation is there any significant difference in the tension exerted by the muscles of these two sorts of animals.

The inferior performance of the muscles of adrenalectomized animals is clearly brought out when one calculates the average "tension-time" for each group. The unit of tension-time may be taken as the kilogram-second, and is defined as the maintenance of a tension of one kilogram for a period of one second. It is calculated as follows:

$$\text{tension-time in kilogram-seconds per gram} = \frac{T_1 + T_2 + T_3 + \cdots T_n.}{W}$$

T_1, T_2, etc., represent the average tension in kilograms developed by the muscle during the first second, second second, etc., of stimulation; T_n is the time when the tension is 10 per cent of the original maximum; W is the weight of the muscle in grams. The tension-time is an expression of the area under the tension curve as recorded by the optical kymograph. The values obtained for the three groups of muscles, together with their standard errors, are as follows: sham-operated, 33.8 ± 2.1; fasted, 31.2 ± 1.6; adrenalectomized, 22.3 ± 1.6.

Discussion. From these results it would seem that adrenal insufficiency does not reduce the functional ability of rat's skeletal muscle for very brief bouts of exercise, but does adversely affect its capacity if the muscle is called upon for a sustained maximal effort. This deficiency is a functional one, for adrenalectomy does not result in a specific atrophy of the muscle; in fact, in the present series of animals, the ratio of gastrocnemius weight to total body weight was exactly the same in both the normals and the adrenalectomized animals; namely, 0.53 gram per 100 grams. The physical or chemical changes in the muscle which might account for the observed functional loss are unknown. Deficiency in carbohydrate stores in the muscle can not account for the reduced capacity, for Britton and Silvette (7) and Silvette (8) have shown that in rat muscle

adrenalectomy does not result in greater reduction of muscle glycogen than does simple fasting. Furthermore, if glycogen storage greatly affected the functional capacity of the muscle, one would expect the muscular performance of fasted rats to be inferior to that of normals, but this is not the case (table 1); it has been established (9) that fasting results in some degree of glycogen depletion in rat's gastrocnemius.

TABLE 1

Effect of adrenalectomy and of fasting on the response of rat's gastrocnemius muscle to tetanizing stimuli

TIME AFTER BEGINNING OF STIMULATION	CONTROLS TENSION	FASTED		ADRENALECTOMIZED	
		Tension	Difference from controls	Tension	Difference from controls
seconds	*grams per gram*	*grams per gram*		*grams per gram*	
1	1944	2075	131 ±97*	1837	107 ±95*
2	1881	2025	144 ±82	1763	118 ±85
3	1819	1969	150 ±79	1663	156 ±77
4	1675	1863	188 ±71	1481	194 ±72
5	1556	1725	169 ±64	1331	225 ±69
10	1144	1163	19 ±55	825	319 ±69
15	863	800	63 ±55	537	326 ±57
20	625	612	13 ±59	369	256 ±59
25	494	450	44 ±65	294	200 ±63

* Standard error.

Whatever the explanation for the reduced performance of the muscle of adrenalectomized animals, it would appear to be a defect present within the muscle before the beginning of stimulation.

SUMMARY

Optical records were obtained of isometric contractions of the gastrocnemius muscles of normal, adrenalectomized and fasting rats. Induction shocks at the rate of 60 a second were used as stimuli, and the response was continuously recorded from the beginning of stimulation until fatigue. The maximum tension exerted at the beginning of the response was the same for all the groups of animals, and the muscles from the adrenalectomized rats fatigued only a little more quickly than the others. The total performance of the adrenalectomized animals, however, was distinctly inferior to the others, because the tension declines more rapidly during the early part of the period of stimulation. It is believed that the functional inferiority of the muscles from adrenalectomized rats reflects a deficiency which existed within the muscle itself prior to the beginning of the stimulation, because under these conditions the muscle is called upon to perform maximally during the entire period of response, and the

level of performance is therefore not dependent upon the efficiency of the circulatory supply during the period of stimulation. The performance of muscles from fasting animals is not inferior to that of the controls; therefore, the functional deficiency observed after adrenalectomy is not due to the general inanition of the organism which accompanies adrenal insufficiency.

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THE EFFECT OF FLUID ABSORPTION ON THE DILUTION INDICATOR TECHNIQUE OF GASTRIC ANALYSIS[1, 2]

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The dilution indicator technique of gastric analysis was recently described in detail and a study was made of the reliability of the various concentrations estimated by this method (Hollander and Glickstein, 1940) with phenol red as the dilution indicator. In another article we reported the results of an application of this method to several human subjects with no obvious gastric pathology, wherein the phenol red test-meal contained alcohol (3.5 per cent or 7 per cent), caffeine solution (6.7 mgm. per liter), or distilled water (Penner, Hollander and Post, 1940). In these studies with humans it was found that the observed concentrations of acid and chloride did not differ significantly from those usually reported in ordinary fractional analyses, but the corrected concentrations were frequently above 165 mN (which corresponds approximately to the tissue fluids in tonicity) and sometimes were even several times this value. Similar hypertonic values have been reported by Wilhelmj, O'Brien and Hill (1936) who ascribed their occurrence to water absorption by the stomach but pursued the matter no further. Since the cause of these hypertonic values may prove to be a major complicating factor in future applications of the dilution indicator technique, we decided to investigate them further and to consider what procedure may be devised to eliminate their occurrence—if this be at all possible.

METHOD. All experiments were performed on dogs in essentially the same way, except for the type of test-meal. The fasting animal was supported in a specially constructed box, a Rehfuss tube was passed into the stomach, fasting contents were removed, the stomach was washed with 50 cc. of the test-meal, and 200 cc. of fresh test-meal were introduced through the stomach tube. Every 15 minutes thereafter the stomach was evacuated completely, the contents mixed, the volume recorded, and all but 15 cc. returned through the tube. This procedure was continued until

[1] This investigation was supported in part by a grant from the Friedsam Foundation.

[2] A preliminary report of this work was presented in the Proc. Am. Soc. Biol. Chem., J. Biol. Chem. 128: xlvii, 1939.

no more fluid could be aspirated. If fewer than 4 quarter-hour specimens were obtained, because of rapid emptying of the stomach, a second (or third) experiment was performed by introducing another 200 cc. portion of the test-meal immediately after the last aspiration and continuing as before.

In all, 64 such experiments were performed with four different dogs. These experiments were divided into four groups, each characterized by a different type of test-meal; i.e., (1) 7 per cent alcohol, (2) distilled water, (3) isotonic NaCl solution (165 mN), and (4) hypertonic NaCl solution (330 mN). Each test-meal contained phenol red (40 mgm. per liter) as dilution indicator. Whereas alcohol was a suitable stimulus to gastric secretion, the water and saline test-meals usually provoked but little secretion, although in occasional experiments (particularly with dog A) the responses were found to be adequate. Since we were particularly concerned with actively secreting stomachs, histamine (in single subcutaneous doses of 0.3 or repeated doses of 0.15 mgm. per kilogram) was used as an auxiliary stimulus to these inadequate test-meals. Although the alcohol test-meal was an adequate stimulus in all cases, a few of these experiments were also supplemented with histamine, in order to be certain that this procedure introduced no gross difference in the results. The first dose of histamine was injected at the time the initial test-meal was introduced. Subsequent injections were given as the situation required.

Each sample of gastric contents was analyzed for free and total acidities, total chloride concentration, and phenol red concentration. The latter was determined by a method previously described (Hollander and Penner, 1940), the other chemical constituents by the standard procedures of this laboratory. The formulae for making the necessary calculations are presented in the Addendum. It should be noted that the equation for the corrected concentration of acid or chloride (C_s) automatically adjusts for the concentration of the acid or chloride initially present in the test-meal (C_t).

Observations. *Series I—Alcohol test-meal.* In the first series of experiments alcohol test-meals were used in order to confirm with dogs our previous observation on humans, concerning the occurrence of hypertonic values for *corrected* concentrations of chloride and acid. The results on a single such experiment are illustrated figure 1. The curves for *observed* concentration (broken line) are both reasonable approximations of the usual findings in fractional gastric analyses, with the highest value attained for acidity or chloride at 135 mN. In fact, throughout this investigation we never obtained a single *observed* concentration value above the isotonic range, 156–175 mN. In contradistinction to this, however, the curve for *corrected* chloride concentration (unbroken line) rises to a peak of more than twice the mean isotonic value of 165 mN, and only one of the

seven points constituting this curve falls below this critical value. No such obvious discrepancy is observable in the corresponding acidity curve, though other experiments of this series occasionally did show high values for this constituent.

In order to obtain some measure of the relative frequency of occurrence of these hypertonic values, the data for the entire series of experiments (14 in all) were analyzed statistically (table 1). From the frequency distribution for the chloride data, it appears that only 16 per cent of all the corrected concentration values fall within the isotonic range, whereas 62 per cent fall above it. In the case of the total acidity data, 8 per cent of

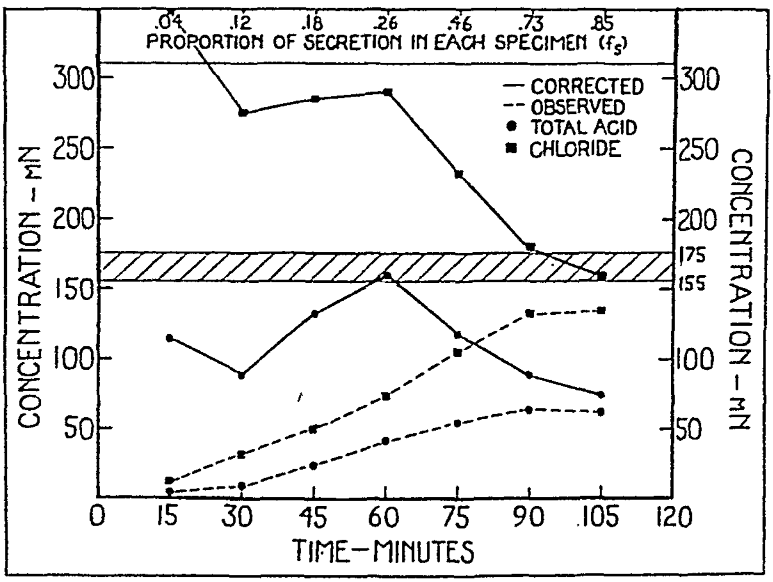

Fig. 1. Acidity and chloride curves with and without correction for dilution by the test-meal.

Experiment L-17. Dog B. Test-meal: vol. = 200 cc.; [alcohol] = 7 per cent; [phenol red] = 40 mgm./liter.

the values are within this range and 5 per cent above it. The frequencies of occurrence of unmistakably hypotonic values (i.e., less than 155 mN) are 22 per cent and 87 per cent for chloride and acid respectively. The difference between these two distributions is also well reflected by their means and their medians.

Series II—Water test-meal (supplemented with histamine). It was next necessary to determine whether the alcohol itself was specifically related to the occurrence of these hypertonic *corrected* concentration values. Hence a second group of experiments was performed with distilled water as the test-meal and histamine as the stimulus to secretion; figure 2 represents an illustrative experiment of this series. Here again the *uncorrected* concentration values appear to be normal whereas the corrected values show

TABLE 1

Frequency analysis of acid and chloride data corrected for test-meal dilution by the dilution indicator technique

(Observations on 4 unoperated dogs—A, B, C, and D)

SERIES	I		II		III		IV	
TYPE OF TEST-MEAL	Alcohol, 7 per cent		Water		NaCl, 165 mN		NaCl, 330 mN	
CONSTITUENT	Acid	Cl	Acid	Cl	Acid	Cl	Acid	Cl
Number (per cent) of specimens in each concentration range:								
155 mN or less	56 (87%)	14 (22%)	38 (62%)	8 (13%)	112 (97%)	39 (32%)	35 (100%)	22 (63%)
156–175 mN	5 (8%)	10 (16%)	13 (21%)	13 (21%)	3 (3%)	82 (66%)	0	13 (37%)
More than 175 mN	3 (5%)	40 (62%)	10 (17%)	40 (66%)	0	3‡ (2%)	0	0
Total*	64 (100%)	64 (100%)	61 (100%)	61 (100%)	115 (100%)	124 (100%)	35 (100%)	35 (100%)
Median	112	188	141	196	119	162	97	151
Mean	108	208	137	215	109	158	93	147
σMean**	5.4	9.0	8.1	14.3	3.2	1.3	5.2	2.8

* Specimens with no free acid were excluded from this frequency analysis.

** This standard error is uncorrected for correlation; such correction would reduce its magnitude in each case.

‡ These three specimens possessed the following corrected chloride values: 177 mN (#55.5), 180 mN (#56.9), 179 mN (#57.5). (See Observations, Series III.)

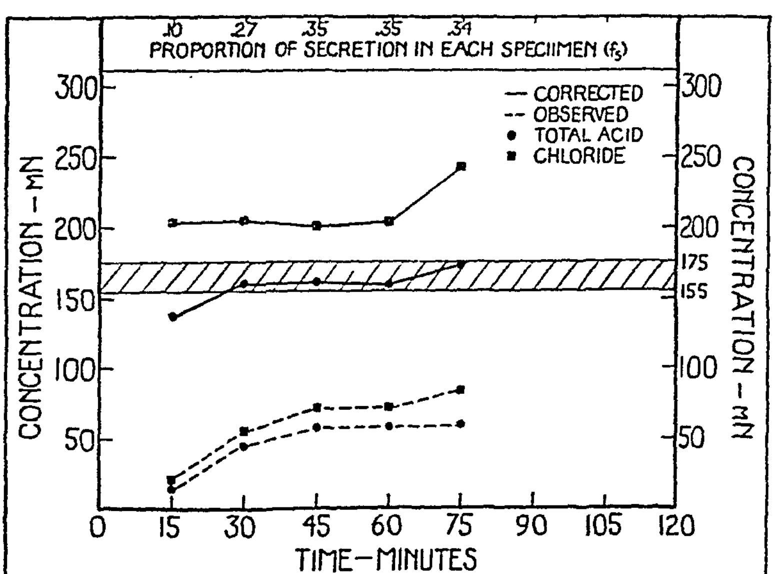

Fig. 2. Acidity and chloride curves with and without correction for dilution by the test-meal.

Experiment L-39a. Dog A. Test-meal: vol. = 200 cc.; water; histamine supplement; [phenol red] = 40 mgm./liter.

characteristics similar to those obtained with the alcohol test-meals. Every single chloride value in the solid line graph is greater than 165 mN, though none of the corrected acidity values of this particular experiment happen to exceed this value. A frequency analysis of the corrected chloride data for all 19 experiments in this group (table 1) reveals the following: 21 per cent of all the values fall within the range 165 ± 10 mN, with 13 per cent below and 66 per cent above this interval. Of the acidity values, 62 per cent are within the isotonic range, 21 per cent below it, and 17 per cent are distinctly hypertonic. These results including the means and medians, are in no way significantly different from those of series I, nor from those previously reported with human subjects.

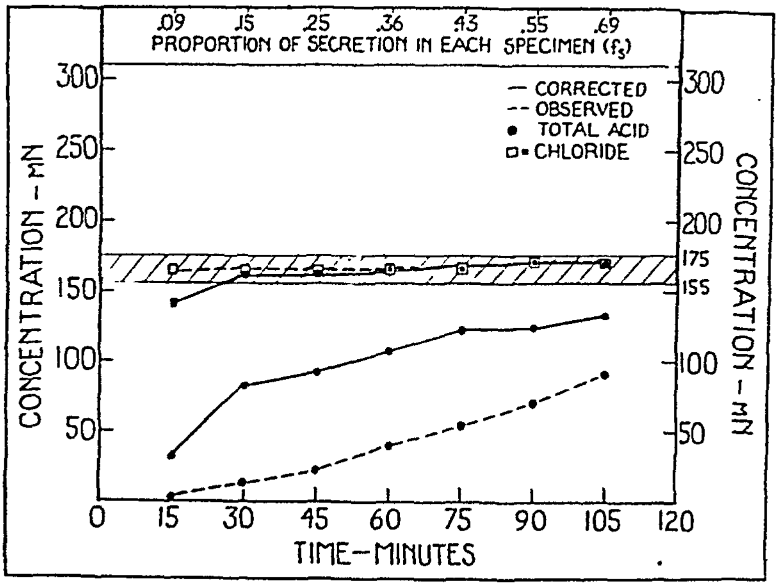

Fig. 3. Acidity and chloride curves with and without correction for dilution by the test-meal.

Experiment L-56a. Dog C. Test-meal: vol. = 200 cc.; [NaCl] = 165 mN; histamine supplement; [phenol red] = 40 mgm./liter.

Series III—Isotonic saline test-meal (supplemented with histamine). Since different hypotonic test-meals give essentially the same result with respect to the occurrence of hypertonic corrected concentrations, we next compared the data from these hypotonic test-meal experiments with those obtained by means of an isotonic test-meal. To this end a number of experiments were performed on the same animals with a test-meal containing 165 mN NaCl, supplemented with histamine injections. An example of this series is presented in figure 3. The curve for observed concentration (broken line) of total acid is grossly the same as in the previous series; that for observed chloride, however, is practically horizontal, indicating a constant concentration throughout the experiment. The cor-

rected chloride concentration graph likewise manifests a considerable degree of constancy, as can be seen from the corresponding (solid line) curve in the illustration. It is noteworthy that not a single corrected chloride value is significantly greater than 165 mN; actually, the highest value obtained in this experiment is 168 mN. The corrected acidity curve for this illustrative experiment likewise never attains a value greater than 165 mN.

The essential characteristics of this individual experiment, in contrast with those employing hypotonic test-meals, are borne out by a frequency analysis of the entire series of 34 experiments (table 1). For total acidity, not a single sample has a corrected value that is hypertonic, and less than 3 per cent of the specimens fall within the isotonic range. The corrected chloride data show only 2 per cent of such hypertonic values whereas the isotonic range contains 66 per cent. Actually, all three of the hypertonic values narrowly miss falling within the isotonic range, as well; their values are 177, 179 and 180 mN. This may possibly be the result of analytical errors only. However, it must be remembered that the constant, 165 mN, is only a statistical approximation to what is in effect an exceedingly variable physiological concept (i.e., isotonicity). Hence it is conceivable that the occurrence of even these few hypertonic corrected concentration values might have been obviated by a slightly different choice of test-meal concentration, relative to the osmotic equilibrium of each animal at the time of experimenting. It appears from these findings that the use of a test-meal containing 165 mN NaCl resulted in the practically complete elimination of hypertonic values for the corrected concentrations, in marked contradistinction to the results obtained with the hypotonic test-meals.

Series IV—Hypertonic saline test-meal (supplemented with histamine). If the isotonicity of the test-meal in series III is the sole or even the major reason for the absence of hypertonic corrected values, it may be expected that a hypertonic test-meal will be similarly effective in suppressing their occurrence. Accordingly, a few experiments were performed with a solution of 330 mN NaCl as test-meal; an illustrative example of these is given in figure 4. The two acidity curves (corrected and uncorrected) of this experiment are essentially the same as the corresponding curves of the experiment with isotonic test-meal illustrated in figure 3; they differ from the latter by no more than other experiments of the isotonic test-meal group differ from each other. The observed chloride curve, which shows a steady decline from 330 mN naturally differs from those previously presented because of the high initial chloride concentration in the test-meal itself. The graph for corrected chloride, however, rises with considerable regularity but never to a value above the isotonic range; the maximum for this experiment is 162 mN. Although the shape of this curve

is in no way typical of all the experiments in this series, the absence of hypertonic values is entirely characteristic. Thus, from the frequency analysis in table 1, it is evident that not a single corrected concentration value for either acidity or chloride is greater than 165 ± 10 mN. In fact, every single acidity value is below this range, in which respect this series resembles the isotonic test-meal group. As for the corrected chloride values, here also the range above 175 mN contains not a single value, whereas 37 per cent of them fall within the isotonic range and the remaining 63 per cent below it—in contradistinction to 66 per cent and 32 per cent for the corresponding values of series III. It is noteworthy that this differ-

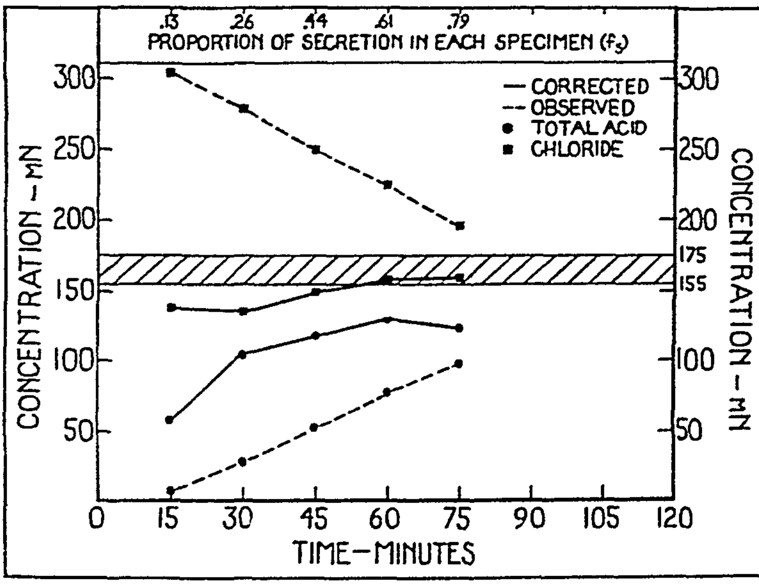

Fig. 4. Acidity and chloride curves with and without correction for dilution by the test-meal.
Experiment L-53. Dog C. Test-meal: vol. = 200 cc.; [NaCl] = 330 mN; histamine supplement; [phenol red] = 40 mgm./liter.

ence in frequency distribution for the chloride values of the two series of experiments is reflected both by their median and their mean concentration values. The medians are 151 and 162 for series IV and III respectively. The corresponding means are 147 and 158 and the difference between them (11) is equal to 3.7 times its standard deviation, without making allowance for correlation. Hence there can be no doubt that the mean chloride concentrations for these two groups of experiments are statistically different. A similar comparison of their acidity values supports this conclusion; the difference between the two means is 16, which is 2.6 times its own standard error. Whether this difference is due to absorption of solute, inhibition of acid secretion, or elaboration of a "dilution secretion," we cannot say at present.

DISCUSSION. It has been shown in the foregoing experiments that the use of a salt-free alcohol solution or of water alone (in conjunction with histamine) as test-meal in gastric analyses by the dilution indicator method frequently results in aberrant corrected concentration values. Taking the two groups of experiments with hypotonic test-meals[3] together (series I

TABLE 2

Frequency analysis of acid and chloride data corrected for test-meal dilution by the dilution indicator technique

(Observations on humans* and dogs with whole stomach pouches or unoperated stomachs**)

SERIES	Humans with unoperated stomachs*		Dogs with whole stomach pouches (group A)**		Dogs with unoperated stomachs (group B)**		Dogs (groups A and B combined)**	
TYPE OF TEST-MEAL	Alcohol, caffeine, water. (No chloride)		Liebig's extract. Total chloride 58–61 mN		Liebig's extract. Total chloride 57–62 mN		Liebig's extract. Total chloride about 60 mN	
CONSTITUENT	Acid	Cl	Acid	Cl	Acid	Cl	Acid	Cl
Number (per cent) of specimens in each concentration range:								
155 mN or less	38 (83%)	16 (33%)	19 (47%)	2 (5%)	54 (95%)	23 (40%)	73 (75%)	25 (26%)
156–175 mN	4 (9%)	5 (10%)	6 (15%)	6 (15%)	3 (5%)	23 (40%)	9 (9%)	29 (30%)
More than 175 mN	4 (9%)	27 (56%)	15 (38%)	32 (80%)	0	11 (19%)	15 (16%)	43 (44%)
Total	46 (100%)	48 (100%)	40 (100%)	40 (100%)	57 (100%)	57 (100%)	97 (100%)	97 (100%)
Median	116	180	162	205	118	158	124	170
Mean	108	211	163	207	106	157	129	178
σMean	6.5	18.8	7.5	5.9	4.1	2.9	4.8	3.9

* Data from study by Penner, Hollander and Post (1940).

** Data from study by Wilhelmj, O'Brien and Hill (1936).

and II), almost 65 per cent of all the Cl values and more than 10 per cent of the acidity values are greater than 175 mN. The incidence of such hypertonic values is equally striking in the studies of Wilhelmj and his co-workers (1936), who employed hypotonic Liebig's extract test-meals in dogs both with whole stomach pouches and with unoperated stomachs.

[3] Although 7 per cent alcohol is about 1.5 molar, there can be no doubt of its "hypotonicity" because of the ready absorption of alcohol by the stomach.

In their experiments, 44 per cent of the total chloride and 16 per cent of the acid chloride values are greater than 175 mN (table 2). Similarly, in our observations with salt-free test-meals on man, we found that 56 per cent of all the Cl values and almost 9 per cent of the acidity values fell above the isotonic range. On the other hand, when the test-meal in the present experiments consisted of isotonic or hypertonic salt solutions the incidence of such abnormal values was practically nil: less than 2 per cent for chloride and zero for acid concentration. A comparison of medians and means for these several groups of experiments supports this evidence. We may conclude, therefore, that there is a specific correlation between the presence or absence of such hypertonic values for corrected concentrations and the tonicity of the test-meal. Their lower incidence for acidity as compared with chloride can be ascribed to the neutralization process, which affects the former but not the latter.

In order to account for the occurrence of these hypertonic values, three possible hypotheses present themselves, based on the following: 1, the elaboration of a hypertonic secretion; 2, the summated effect of random analytical errors in the phenol red, chloride, and acidity determinations, and 3, the gastric absorption of water, which would increase the apparent concentrations of all solutes in the gastric fluid as determined by this method.

Of these three explanations, that based on a hypertonic secretion is least tenable, if only because such a secretion is unknown throughout the alimentary canal. The second explanation, based on the analytical errors of the method, merits more consideration. We have shown elsewhere (Hollander and Glickstein, 1940) that even though the standard errors of individual chemical analyses be suitably low, the mathematical manipulations of the dilution indicator method may combine these errors with considerable magnification. Thus, under certain conditions of test-meal concentration, C_t, and proportion of secretion in the gastric fraction, f_s, the standard error of a corrected concentration value, C_s, may be 50 per cent of that C_s-value, or even considerably greater. Under most conditions, and particularly when f_s is greater than 0.2[4], these errors remain within a limit, which, though large, is acceptable for our present purposes (i.e., a maximum standard error of 20 m.eq. per liter). Now, in order to determine whether the analytical errors alone are sufficient to account for the aberrant concentration values, we have studied the correlation of low and high f_s-values (i.e., less than and greater than 0.2) as compared with the incidence of hypertonic and non-hypertonic Cl-values in all our data. The results, arranged in two groups for series I and II and series III and IV

[4] An f_s-value of 0.2 means that the specimen of stomach contents contains two parts by volume (20 per cent) of secreted fluid and eight parts (80 per cent) of residual test-meal.

respectively, are summarized in table 3. The extremely low values for the coefficient of mean square contingency (0.06 for both groups of data) demonstrate the almost complete absence of correlation between tonicity of corrected Cl value and magnitude of f_s; i.e., the distribution of these gastric samples among the four cells falls just short of being entirely random. In other words, low f_s values (which are most likely to produce the greatest analytical errors) are associated no more frequently with hypertonic corrected concentration values than with non-hypertonic values. Hence, it may be concluded that the influence of analytical errors is but a minor one in this connection.

TABLE 3

Independence of tonicity of corrected chloride values and proportion of secretion (f_s)

	NUMBER OF f_s VALUES			COEFFICIENT OF MEAN SQUARE CONTINGENCY (PEARSON'S)
	<0.2	$\geqslant 0.2$	Totals	
Series I and II—Hypotonic test meals				
Number of Cl values:				
Hypertonic	26	54	80	$C = \sqrt{\dfrac{\chi^2}{N + \chi^2}}$
Non-hypertonic	12	33	45	$= 0.057$
Totals	38	87	125	
Series III and IV—Non-hypotonic test meals				
Number of Cl values:				
Hypertonic	1	2	3	$C = \sqrt{\dfrac{\chi^2}{N + \chi^2}}$
Non-hypertonic	32	124	156	$= 0.062$
Totals	33	126	159	

We are left, therefore, with the third hypothesis—that based on fluid absorption. This explanation, together with some supporting evidence, was advanced by Wilhelmj, O'Brien and Hill (1936), but these authors associated the absorptive process with variations in acidity rather than in osmotic concentration of the stomach contents. Although early workers, Edkins (1892) and Von Mering (1893), found no evidence for gastric absorption of water, Faitelberg (1930) and Sleeth and Van Liere (1937), by means of improved techniques, were able to prove that this phenomenon does occur. Hence, such an explanation for the occurrence of hypertonic corrected concentrations is clearly a tenable one. It may be argued that the rate of water absorption under our conditions of experimentation is likely to be very small, and quantitative evidence in support of this contention can be found in the several investigations just cited. However, by means of the mathematical relations developed previously (Hollander

and Glickstein, 1940) it can be shown that the influence of such a small water loss on the corrected concentration values may be very great, because the entire effect of this loss operates in diminishing the apparent volume of secretion in the gastric specimen but not at all the volume of residual test-meal. *In other words, the dilution indicator technique involves the tacit assumption that the test-meal retains its identity (original composition) throughout the experiment, no matter what changes occur in the stomach contents as a result of secretion and absorption.* Such a decrease in the apparent volume of secretion must yield an increase in the apparent concentration of all its solutes provided, of course, that these solutes do not undergo differential absorption. By way of illustrating this point without resort to a technical mathematical analysis, it has been shown in a purely hypothetical situation (Penner, Hollander and Post, 1940) that parietal secretion with a chloride concentration of 165 mN is capable of yielding an apparent corrected chloride concentration of 330 mN (i.e., an error of 100 per cent) solely as the result of the absorption of as little as 5 cc. of fluid during the period of observation.

Further evidence in support of the absorption hypothesis, however, is afforded by the fact that these excessively high values are essentially non-existent in the two series of experiments employing isotonic and hypertonic test-meals. Whether such test-meals eliminate fluid absorption completely or merely reduce its speed to a very low rate is a moot question, but the simultaneous elimination of hypertonic corrected concentration values indicates a probable relation between these latter values and such absorption. Furthermore, Wilhelmj (1938) has found "a fairly good number of experiments on whole stomach pouches in which there was a rise in the . . . concentration of the phenol red to values of 114 and 120 per cent of the meal," which he accounted for by a differential absorption of water and concentration of the non-absorbable indicator. We have never observed this phenomenon in our own work, but in the course of several of our experiments (L-36, 39, and 46) we have noted that the usual progressive drop in phenol red concentration was followed by a secondary rise, suggestive of an absorption rate which exceeded the rate of secretion during the latter part of the experiment.

In view of the foregoing observations, there can be little doubt that 1, hypertonic corrected concentration values are wholly erroneous, and do not reflect the true composition of the secretion; 2, they are traceable chiefly to fluid absorption by the gastric mucosa and only in small part to the analytical errors of the dilution indicator technique; 3, where they occur as a result of fluid absorption, *all* the other corrected concentration values of the same experiment must also be considered erroneous, and 4, their occurrence can be prevented almost entirely by the use of an isotonic salt test-meal. The addition of secretagogue substances (as contained in

Liebig's extract) to such a test-meal ought not to affect the results any more than a subcutaneous injection of histamine given parallel with the test-meal. Alcohol, however, should not be added to the test-meal as a gastric stimulus in such experiments, because it may be absorbed at a much greater rate even than water. Another complicating factor which may be present is the absorption of acid and salt, in which case it will also contribute errors of indeterminate magnitude in the use of the dilution indicator method.

Fluid absorption in the course of experiments employing the dilution indicator technique may seem to be of minor physiological importance, because of the low absorption rate. Also, it may be contended that the same absorption process obtains in ordinary gastric analyses without consequential effect on the (uncorrected) acidity and chloride curves. The significance of the present investigation, however, lies not in the mere existence of such absorption but in the fact that *its occurrence will give rise to values calculated for the fraction of the test-meal, the fraction of secretion, and the corrected concentrations of the constituents of the secretion which are erroneous.* In consequence, any data except total amounts of solute which may be derived from these erroneous values (e.g., the volume of secretion contained in a gastric specimen or the concentration of chloride and base in the non-acid secretion) are likewise open to error. Since Wilhelmj and his co-workers have based many of their diverse studies on the dilution indicator technique with phenol red and Liebig's extract test-meals of low salt concentration, their observations are open to question on this score and must be repeated with isotonic salt test-meals before all their conclusions can be accepted.

SUMMARY AND CONCLUSIONS

1. Gastric analysis experiments, based on the dilution indicator technique with phenol red, were carried out on unoperated dogs. When the test-meal was alcohol, or water supplemented with histamine, it was found that many of the corrected concentration values for total acid and total chloride were hypertonic. Such abnormally high values had been observed previously by us in humans and by Wilhelmj and his co-workers in dogs. In all these instances, the test-meals were either salt-free or contained salt in less than isosmotic concentration.

2. When isotonic or hypertonic salt test-meals were used, no such abnormally high values were obtained, except for a very few which might be related to errors in the chemical analyses.

3. For this and other reasons, these high values are ascribed only in small part to the analytical errors of the dilution indicator technique, but chiefly to absorption of fluid by the gastric mucosa.

4. Hence, these hypertonic values are wholly erroneous and do not re-

flect the true composition of the secretion, as has heretofore been assumed. In any such experiment which yields hypertonic corrected concentration values not explainable by analytical errors, it must be presumed that fluid absorption has taken place, and *all* the corrected concentration values (even those *not* hypertonic) must be considered spurious.

5. We believe that the dilution indicator technique should be used only with isotonic test-meals. This will minimize the errors due to water absorption and may even eliminate them entirely.

6. Any experiments by the dilution indicator method, designed to yield *volume or concentration data* concerning the various constituents of the secretions of the stomach, are open to question if hypotonic test-meals have been used. Such investigations must be repeated with isotonic test-meals before all their conclusions can be accepted. Conclusions concerning *amounts* of the several components, however, are not subject to this error unless the individual components undergo absorption.

ADDENUM. The following formulae are those used in the dilution indicator method of gastric analysis; for their mathematical development see the previous report by Hollander and Glickstein (1940).

$$f_t = \frac{P_0}{P_t}$$

$$f_s = \frac{P_t - P_0}{P_t}$$

$$C_s = \frac{C_0 - C_t f_t}{f_s} \quad \text{or} \quad C_t + \frac{C_0 - C_t}{f_s}$$

where C_s = the concentration of any component, as acid or chloride, in the mixed secretion; i.e., the *corrected concentration* value.

C_t = the concentration of this same component in the test-meal.

C_o = the concentration of this same component in any gastric specimen, i.e., the *observed concentration* value.

P_t = the concentration of phenol red in the original test-meal.

P_o = the concentration of phenol red in the gastric specimen.

f_t and f_s = the proportions by volume of test-meal and mixed secretion in the gastric sample; i.e., $f_t + f_s = 1$.

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INTERACTION OF MEDULLATED FIBERS OF A NERVE TESTED WITH ELECTRIC SHOCKS

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In 1932 Blair and Erlanger described experiments performed with the object of ascertaining whether activity of some of the medullated fibers of a nerve trunk *increases* the excitability of inactive medullated fibers in the same trunk. The results in *normal* nerve were negative. Since then, Jasper and Monnier (1938) have demonstrated the transmission of the impulse from one nerve to another in juxtaposed preparations of crustacean (nonmedullated) nerve, but only when the receiving nerve is on the verge of spontaneous discharge.

More recently Katz and Schmitt (1939, 1940), employing two adjacent giant fibers of *Carcinus*, have demonstrated a triphasic effect on the excitability of one fiber by a propagated disturbance in the other, consisting in succession of 1, a fall (beginning before the arrival of the spike); 2, a rise, each amounting to about 20 per cent of the threshold, followed by 3, a much lower and longer fall. They attribute this excitability sequence to the current emanating from the active locus which reverses twice as the latter progresses.

Under the circumstances we have reinvestigated the interaction of medullated fibers employing this time a method that would disclose not only increased response, which was all our previous method could disclose, but decreased response also. The experiments have disclosed that there is induced an early increase in response which in normal nerve, however, is completely masked through shunting of the testing shock by the conditioning fibers, whose resistance is low while they are in a state of activity.

METHODS. In principle the method consists of starting a maximal spike, the conditioning action potential, along one dichotomous branch of a large mixed nerve. At appropriate intervals thereafter a submaximal stimulus is applied to the parent nerve and the resulting response is recorded from the other branch. The change in the height of the response as the testing stimulus falls, in successive trials, in every relation to the conditioning action potential expresses the effectiveness of the testing stimulus as modified by the conditions of the experiment.

The sciatic nerve of the green frog (*Rana pipiens*), with attached peroneal and tibial branches, was the most frequently used preparation. The preparations were usually equilibrated in Ringer's solution for one or more days at 5°C. and during experiments were mounted in a moist chamber, in air, at room temperature.

Electrode arrangements. The preparation (see figs. 1 and 2) usually was suspended by the two branches containing the conditioning and the conditioned fibers, which were held apart by silver electrodes. A large silver plate (13 x 25 mm.) was placed against the nerve roughly centered on the tibial-peroneal bifurcation. In some experiments the plate was placed horizontally so that it would, by capillarity, hold on its upper surface a pool of Ringer's solution into which the part of the nerve on the plate could be immersed. The plate was grounded and acted as the cathode of the conditioning stimulus applied to the tibial, as the cathode of the testing stimulus to the sciatic and, interchangeably, as one amplifier lead (ground) for the recording of the conditioning spike in the sciatic nerve or of the conditioned spike in the peroneal nerve. Other silver electrodes appropriately placed, completed the above circuits. This simple electrode arrangement so completely eliminated shock escapes that it usually was necessary to connect an aerial to the amplifier input in order to introduce a shock artifact when a record of the instant of stimulation was needed. Since there is only one ground connection to the nerve, interaction is minimized of the various circuits through ground surges, such as occurred in the earlier experiments in which multiple electrodes took the place of the plate. Under the earlier conditions interaction of the stimulating circuits sometimes occurred, but such interaction involved no conduction delay and could easily be identified and distinguished from the effect of the conditioning response, which appeared only after a significant conduction time.

Recording. The activity of the nerve has been recorded by leading the action potentials through the electrodes mentioned above into an amplifier which drives an electron oscillograph. Changes in the response of the fibers at the tested locus in the sciatic express themselves as changes in the height of a submaximal spike initiated there and recorded from the conditioned fibers in the peroneal. In practice the maximum conditioned spike from the peroneal has been brought to a selected amplitude on the oscillograph by varying the amplification. Then the change in height with the change in voltage of the testing shock is observed throughout the submaximal range and the shock strength fixed at such a level that small changes in the stimulating voltage produce maximum changes in spike height. Usually the strength selected has produced a spike which is somewhat less than half maximal in height. This fixed shock is applied to the tested locus of the sciatic at variable intervals after a maximum

conditioning action potential is initiated in the conditioning nerve, and the effect of this action potential on the peroneal fibers as conditioned by it at the sciatic locus manifests itself as changes in height of the action potential initiated there and recorded from the peroneal nerve. In some of the later experiments the deep and the superficial tibial nerves were employed as the conditioning and the conditioned branches, respectively.

As many responses to the testing stimuli as could be distinguished from each other, scattered over the temporal range under observation, have been photographed as multiple exposures on a single film (see figs. 2 and 3), and from seven to ten of these films have been used in the construction of each of the response curves. Chance variations in excitability have thus been reduced to a minimum.

Stimulation is accomplished by short shocks (RC $\approx 1 \times 10^{-4}$ sec.) from independent gas discharge tube stimulators, delivered to the nerve through low capacity, shielded transformers. The cathode ray sweep circuit and the two stimulators are activated through delay circuits by a master oscillator usually discharging at the rate of about 70 per minute.[1]

RESULTS. At first the response curves derived from different points along a preparation varied quite unaccountably, though the same point continued to yield its original record. Eventually, when the conditions determining the variability came to be understood, reasonably constant and predictable pictures could be obtained. Most of the disturbing variations in the response curve were found to be referable to polarization of the tested locus by action and injury currents acting through stray circuits permitted by branches, cut and uncut, or by some other discontinuity in the nerve's structure.[2] A potent source of distortion, for example, has been the tibial-peroneal junction. It was not realized, at first, that the junction which presumably determined the distortion is not, or not to any degree, the crotch between the two nerves, but lies rather somewhere proximal[3] to this point, probably where the connective tissue septum ceases to be an effective electrical barrier between the two sets of nerve fibers. Between that point and the ground there will be an additional path through which could flow a part of the current determined by the potential drop between the confluence and the grounded lead. During the period of such current flow, which would be coincident with the passage of a spike, the excitability of the fibers at the tested locus would be altered, —elevated if the flow polarized the tested locus cathodally, depressed if it were polarized anodally. In order to minimize this particular action

[1] For further details concerning method consult the legends to the several figures.

[2] For a discussion of the conditions that may distort action potentials as led from the nerve's surface consult Bishop, Erlanger and Gasser (1926).

[3] Terms of direction refer to the normal anatomical relations of the sciatic nerve, not to the direction of impulse transmission.

the preparations eventually were mounted with the tibial-peroneal crotch on the grounded plate as far from the testing cathode as was consistent with securing a clean, unbranched stretch of sciatic of sufficient length beyond (central to) the grounded lead. Generally the crotch has occupied a position about 10 mm. distal to the tested locus; and then there was left a clean stretch of sciatic something over 10 mm. in length between the tested locus and the first of the larger thigh branches. This distance proved to be just about sufficient to reduce to insignificance the disturbing

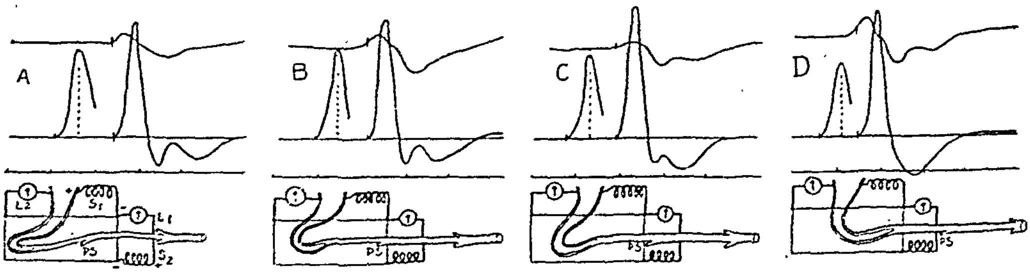

Fig. 1. Illustrating some of the variations in the configuration of the response curve referable to structural and injury factors.

The position of the proximal portion of the sciatic nerve was changed for each trial as indicated in the diagrams. The distance between the terminals of S_2 was 7.4 mm. PS = peroneal stump.

Differences in the configuration of the response curves as the nerve is moved are quite obvious. It would not be profitable to account specifically for them.

The following applies to all figures. From above downwards are shown the response curve, the relation to it of the conditioning spike (the second in each case), the time in msec., and a diagram of the circuit in relation to the preparation as used. The nerve length is drawn to scale. S_1 = conditioning stimulator; L_1 = lead for recording conditioning spikes; S_2 = testing stimulator; L_2 = lead for recording the conditioned responses. The grounded lead is a silver plate outlined by lighter lines. Wires are indicated by the heavier lines. The crest of the first spike marks the arrival of the conditioned spike at L_2. The second is the conditioning spike recorded at the conditioned locus through L_1. In the diagrams it is set with the escape of the shock initiating it on the crest (extended to the base line) of the first spike; the beginning of the second spike (indicated by the mark on the response curve) therefore marks the time conditioning begins at the tested locus.

effects of injuries, branches, etc. in the proximal region on excitability at the tested locus. Some notion of the complications encountered when precautions are not taken to work under conditions that minimize these sources of distortion may be obtained through inspection of the response curves (fig. 1) derived from one of the experiments performed with a view to ascertaining sources of distortion.

When every indicated precaution is taken to avoid these disturbing factors the response curves have the configuration seen in figures 2, A and B, and 4, A. At the instant of the arrival of the conditioning spike at the tested locus the response elicited from the test fibers diminishes

and it then remains low for something less than the duration of the spike, when the latter is recorded monophasically. In the trough of curves of this type, with rare exceptions, an upward bowing can be detected beginning before the conditioning spike attains its crest. The response then grows, rapidly at first, along a curve which becomes convex upwards and passes above the normal level before it begins to bend toward it. Since the periods of observation succeeding the arrival of the spike at the tested locus have not exceeded about 4 msec. nothing is known regarding the final stages of the response cycle.

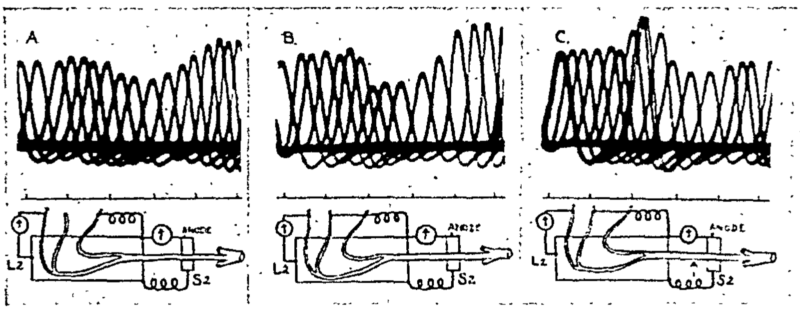

Fig. 2

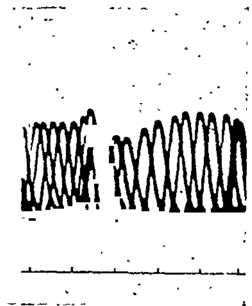

Fig. 3

Fig. 2. Illustrative records of response changes, reproduced in approximately the photographed size. The crests of the spikes outline the response curve, and 7 to 10 such records, enlarged 4 times, are employed in the construction of each of the curves such as are copied in the other figures.

A, conditioning through the peroneal; B, conditioning through both the peroneal and the deep tibial; C, same as B, but after killing the nerve at the point indicated by the arrow, 5.8 mm. from conditioned locus. The conditioned fibers conduct into the superficial tibial in all cases. The cathode-anode distance of S_2 is 8.7 mm.

Fig. 3. A record from another preparation showing a slightly different type of response curve. Superficial tibial conditioned via deep tibial. An initial increase in response precedes the arrival of the spike at the conditioning locus, possibly an effect produced by the tibial-peroneal junction resting on the plate.

Some of the more frequently observed departures from this typical response may now be considered. Quite commonly the response begins to grow 0.2 to 0.4 msec. before the conditioning spike arrives at the tested locus (fig. 3). This rise usually can be attributed to the discontinuity at the tibial-peroneal confluence. If 30 m.p.s. be taken as the conduction rate, it would appear that a potential fall developing at such a discontinuity can affect the tested locus when the two are separated as widely as 6 mm. A much rarer departure from the rule has been a decrease in response beginning slightly before the conditioning spike leaves the grounded lead. Under the latter circumstances the arrival of the spike at the tested locus starts the characteristic, the more rapid, decline seen in the typical response curves. When the true tibial-peroneal confluence happens to be distal to the tested locus, but not too distant from it, the depressed portion of

the response curve may exhibit one or two prominent elevations which, however, rarely rise to the basal level. We were unable to account for these elevations before it became apparent that the true confluence is situated central to the tibial-peroneal crotch.

Crushing the nerve central to the tested locus, and within a range of about 10 to 12 mm., alters the configuration of the response curve. In figure 4 it is seen that the curve is unaltered when the nerve is killed 17.0 mm. proximal to the tested locus (B), and that killing at a distance of 6.6 mm. (C) completely changes it. A similar alteration, but of greater

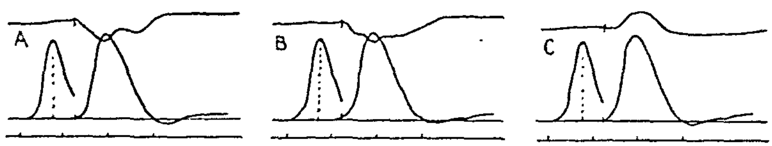

Fig. 4. A set of typical response curves.
A, separation of S_2 electrodes 19.0 mm.; B, the same but after killing at 17.0 mm.; C, separation of S_2 electrodes 8.1 mm.; killed at 6.6 mm.

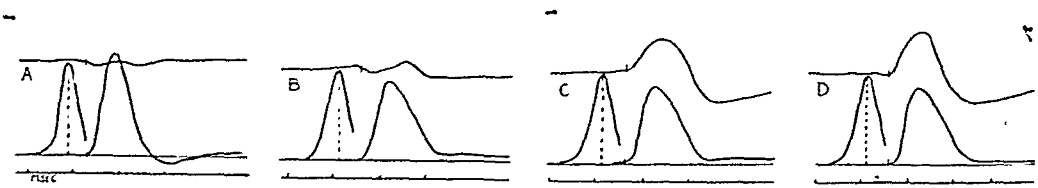

Fig. 5. Illustrating the effect of killing at different distances central to the conditioned locus.
The interelectrode distance for S_2 is 11.1 mm. A, before killing. This is the smallest response ever obtained from the frog's sciatic. The up and down effects must almost exactly have balanced. B, after killing at 8.5 mm.; C, at 5.6 mm.; and D, at 1.3 mm. distal to the tested locus. D is one of the rare instances in which, in a killed preparation, there is an initial "down" effect beginning *before* the spike arrives at the tested locus.

ultimate magnitude, is seen in figure 5. Here the change is slight when the distance between the injury and the lead is 8.5 mm. (B); it is much greater when the nerve is killed at 5.6 mm. (C); and greater still, but only slightly, at 1.3 mm. (D). The alteration in configuration consists of the replacement of the period of reduced response (practically absent in fig. 5, A) with one of increased response, and of the conversion of the upward convexity of the ending of the curve into an upward concavity approaching the normal level from below as the observation terminates.

We have next to account for the configurations of these response curves. In the first place it should be stated that when every precaution is observed to avoid complicating circumstances moving the anode of the testing circuit is without effect on their configuration. All of the processes that

change the configuration apparently must act, either directly or indirectly, through the locus under the cathode. Here it might be added that currents referable to action potentials or demarcation potentials acting through stimulating circuits have, in our experience, been without appreciable effect.

The trough in the curves derived from *uninjured* nerve must, we feel, be attributed to shunting of the stimulating current lines by a lowering of the resistance of the conditioning fibers with their entrance into activity. Since experiments we have done show that the depth of this part of the curve does not increase in proportion to the length of nerve subtended by the stimulating electrodes (the changes in length were made in short steps) it would seem to follow that changes in transverse resistance are in the main responsible for this effect.

Little is known regarding the configuration of the resistance changes associated with activity under the conditions of our experiments. Cole and Curtis[4] have shown (1939) that in single fibers the resistance decreases rapidly *after* the start of the spike and then increases more gradually toward the normal level. With the temporal dispersion that obtains in conducted multifiber spikes the decrease in resistance would be less steep than the rise of the recorded spike and less steep than in single fibers. We will assume, in the absence of specific information, that the resistance curve follows in general the configuration of the excitability cycle of nerve that has responded to a stimulus.

Now our "normal" nerves have recovered from refractoriness through a period of supernormality which has reached its maximum in about the same time as the response curves derived from normal preparations pass through their maximum during recovery. When the tested locus is under the influence of a demarcation current it is cathodally polarized, and it is known that cathodally polarized nerve in recovering from refractoriness does not pass through a supernormal period. Under these circumstances the resistance would be expected to return to the normal level without passing through a supernormal phase. On this basis it becomes possible to refer not only the earlier of the more prominent features of our "normal" response curves, but also the later, to variations in the shunting of the stimulating current used for testing, by resistance changes in the conditioning fibers.

It is possible, however, to account for the last phase of the response curve perhaps equally well on the basis of what is known regarding the influence exerted by polarization on the excitation cycle initiated by a *subthreshold* stimulus. If the conditioning spikes in the present experiments act as subthreshold stimuli to the tested fibers then (Blair)[5] when the latter are

[4] A bibliography of the subject is given by these authors.

[5] Cf. Erlanger (1939).

"normal" the excitability of the fibers would not at any time in the resulting cycle fall below the normal level. If, however, the tested locus were cathodally polarized, as by a demarcation current, the excitability following the period of latent addition would fall below the normal level and would remain subnormal for at least 3 msec. It is obvious then that through either set of assumptions the state of polarization of the tested locus could determine the configuration of the terminal phase of our curves.

But if the conditioning spike acts as a subthreshold stimulus the first response to it should be an increase in the excitability of the fibers it conditions. In *normal* nerve, however, the arrival of the action potential at the tested locus coincides with a decrease in response and it therefore becomes necessary to conclude that if there be any excitability increase due to latent addition it ordinarily is concealed by a simultaneous decrease in the effectiveness of the testing stimulus.

There is another consideration, besides those already mentioned, that suggests the inference that at least those parts of the response curve that coincide with the spike of the monophasic action potential are the resultant of two oppositely directed influences. It has been seen that practically invariably that portion of the curve gives evidence of an upward bulge with peak a bit later than the crest of the monophasic spike (see fig. 4, A). This might be expected if this part of the curve were determined by the addition of two curves oppositely directed, one downward, having somewhat the shape of the curve of increased electric conductance (Cole and Curtis, 1939), the other of an upright monophasic spike lower in amplitude than the former. If the crest of the spike appeared while the resistance is decreasing rapidly, it would be displaced to the right with respect to the spike crest, just as is the upward bulge in our curves.

But perhaps the best evidence of the presence of an underlying excitation effect in the "normal" nerve is supplied by the gradual transformation of a "normal" response curve beginning with apparent depression, into one in which enhancement replaces the depression, by bringing a killed region closer and closer (within limits) to the tested locus (see fig. 5). This transformation very largely is referable to the increase in excitability determined at the tested locus by cathodal polarization. Since excitation by subthreshold stimuli is a function of the strength of the stimulus, it must be supposed that the action potentials that produce no obvious upward bulge in the "normal" response curve are nevertheless exerting their proportional effects in that direction.

Some conception of the magnitude of the changes in stimulus effectiveness that are determined by a passing spike can be obtained by comparing the variation in the height of the conditioned spikes elicited by a constant testing stimulus, with the variation in the height of the spikes in the same fiber group elicited by stimulating with shocks of known strength. It

is scarcely necessary to add that the values obtained will vary with changing conditions; that they will be qualified by the number of the conditioning fibers, the intimacy of their mixture with the fibers conditioned, the height of the composite spike as determined by the degree to which the axon spikes are in phase, and so on.

In normal nerve, then, we are dealing with a degree of excitation by the action current that is concealed by shunting of the testing current. The combined result is depression and this has been the equivalent of a reduction in shock strength of over 10 per cent.

The increase in excitability becomes apparent only when the tested locus is under the influence of a demarcation current. The largest increase we have seen has amounted to an equivalent increase of 26 per cent in shock strength. Since this increase is based on the depression produced by the shunting action of the conditioning fibers, the total increase in excitability must be equal to something more than the sum of the two values given above, or to an effective change of over 36 per cent of the shock strength.

Discussion. The curves derived by us from *normal* nerve show no resemblance to those constructed by Katz and Schmitt. On the other hand, from loci under the influence of a demarcation current, and therefore highly excitable, pictures have been obtained, though exceedingly rarely, that match exactly, in sign and in temporal correlations, those derived by them through a study of the action of one giant fiber upon another. The closest match is seen in figure 5, B. Here, as in Katz and Schmitt's curves, this curve begins in a direction indicative of a decrease in excitability that starts before the conditioning spike arrives at the tested locus. As in all other of our curves obtained under these conditions, this one then conforms with those obtained in experiments on giant fibers. Now it may be relevant to note that in this particular case (see fig. 5, A) the changes in stimulus effectiveness exhibited by the normal nerve were unusually slight. This situation might signify that in this particular instance the excitability changes were so great that they almost completely masked the shunting action, and therefore that the pictures obtained when the excitability of the tested locus was increased by the demarcation current were qualified still less by the resistance factor. If these actually were the conditions, it would be necessary to conclude that the sequence of the excitability changes induced by the action of a spike in one fiber on a contiguous fiber is the same in both medullated and nonmedullated fibers when resistance changes associated with activity of the conditioning fiber, when medullated, are discounted. We have seen, however, that there are other factors involved in the production of these response curves than the excitability changes determined by current flow from the active fiber. What the relative significance of all of the factors may be remains to be determined. It is quite possible that resistance changes may be

found to be much less significant in nonmedullated than in medullated fibers.

Observations on the phrenic nerve. A few similar observations have been made with the dog's phrenic nerve, desheathed, employing the same general technique. The conditioning maximal stimuli were applied to one of the trunks of origin of the nerve, the testing submaximal stimuli to the nerve well beyond the union of the trunks, and the variations in the height of the spike elicited by the latter stimuli were followed in the other trunk of origin. In previous work, employing a method which could indicate only an increase in excitability or an increase in stimulus effectiveness, the conclusion was reached that a passing spike does not *increase* the excitability of adjacent fibers, though an increase was demonstrable after supplying conditions that might have caused a demarcation current to flow through the tested locus (Blair and Erlanger, 1932).

The present experiments on the phrenic nerve confirm the earlier ones and show that, as in frog's nerve, a passing spike actually lowers the response. The pictures, though, have changed unaccountably with shifting of the anode of the testing circuit. Based on our experience with frog's sciatic, it would seem reasonable to assume that the desheathed phrenic is not as uniform a structure as it appears to be,—that the variability of the pictures with change in electrode position is referable to local injuries and discontinuities resulting from the removal of the sheath. The difficulty could not have been avoided by leaving the sheath on, since the surface of the sheath also is ragged. The amplitude of the response changes in the phrenic has been very much lower than in frog's nerve. The reason for this has not been investigated.

Functional significance. That the lowering of stimulus effectiveness seen in these experiments could be of any physiological significance in the normal functioning of nerves seems highly improbable.

In the central nervous system, however, the situation might be different. The final ramifications of fibers in the nerve centers are so closely packed that interaction could readily occur between them. Assuming that synaptic transmission can be accomplished electrically, it is conceivable that such transmission would be aided by poor electrical conductivity of surrounding tissues (which would be their state during inactivity) and hindered or, may we say, inhibited, by good electrical conductivity (during activity of surrounding tissues). Since in the central nervous system summation of impulses usually is required for synaptic transmission it is obvious that even a very slight variation in shunting of current lines might make the difference between transmission and failure of transmission.

In this connection attention may again be called to the enormous increase in the efficacy of issuing current lines as stimuli that results through cathodal polarization (by demarcation currents) of the fibers they act on.

Though the fact is as old as the rheoscopic nerve-muscle preparation, this demonstration of its relation to from-fiber-to-fiber action in a nerve trunk reminds us of the important rôle varying degrees of polarization could play in the functioning of the central nervous system.

SUMMARY

A method is described of ascertaining graphically the changes in the effectiveness of a stimulating current on inactive medullated fibers of a nerve trunk when other medullated fibers of the trunk are conveying impulses.

In "normal" nerve (frog's sciatic) as the multifiber spike passes by the tested locus the main features exhibited by the curves are a depression, about coincident with the monophasic conditioning spike, followed by an elevation preceding the return to normal.

In nerve "killed" not further than 10 to 12 mm. from the tested locus the depression is replaced, to a degree depending on proximity to the injured locus, by an elevation and this is followed by depression. In recovering, the latter does not pass above the normal (resting) level during the period of observation.

This development of a spike in the response curve is attributed primarily to an increase in excitability induced by the demarcation current; and it is inferred that the absence of a "spike" in the "normal" curve is the result of masking effected by a concurrent reduction in the resistance of the conditioning fibers, resulting in the shunting of the testing current.

The possibilities are considered *a*, that resistance changes might be responsible also for other features of the response curves, and *b*, that latent addition might enter as a factor.

It is suggested that the "resistance cycle" might play a rôle in synaptic transmission in the central nervous system.

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THE RÔLE OF LACTIC ACID IN THE MOVEMENTS OF POTASSIUM

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A large number of conditions are now known in which potassium enters the blood from the tissues. Some of these have been reviewed recently by one of us (Fenn, 1940), and it was pointed out that frequently lactic acid appears in the blood at the same time as potassium. The possibility was suggested, therefore, that the potassium enters the blood in the form of potassium lactate. In this paper it is proposed to present a number of recent experiments which bear upon this question. They tend to show that there is no necessary connection between the lactic acid and the potassium.

METHODS. Potassium in the plasma was analyzed by Wilde's (1939) modification of the Shohl and Bennett method and lactic acid in whole blood by Koenemann's (1940) modification of the method of Miller and Muntz. The pH was measured with a Beckman pH meter using a glass electrode of the type described by Pickford (1937) which permits samples to be withdrawn from under a layer of oil or from a tonometer after equilibration with 5 per cent CO_2 and measured without exposure to air. Cats were anesthetized with dial. Other details are described in what follows or by Fenn, Wilde, Boak and Koenemann (1939) or Fenn, Koenemann and Sheridan (1940).

RESULTS. *Asphyxia.* As shown by Civin and Cattell (1938) and others there is a rise in plasma potassium when a cat is asphyxiated by clamping the trachea. A similar experiment on a cat with additional information concerning the pH of the arterial blood and its lactic acid concentration is plotted in figure 1. Four successive periods of asphyxia produced in this way are shown. In each case there was a sharp rise of potassium accompanied by a smaller rise of lactic acid.[1] At most, therefore, only a part of

[1] The smaller rise of lactic acid compared to potassium might possibly be due to a more rapid removal from the blood by inactive tissues. To test this point in one experiment we injected 5 cc. of 0.19M potassium lactate intravenously into a cat and analyzed the plasma at intervals thereafter for both K and lactate. There was some indication 8 or more minutes after injection of a more rapid decrease of lactic

the potassium increase could be associated with lactic acid. At the same time the pH of the arterial blood became more acid while the pH of the

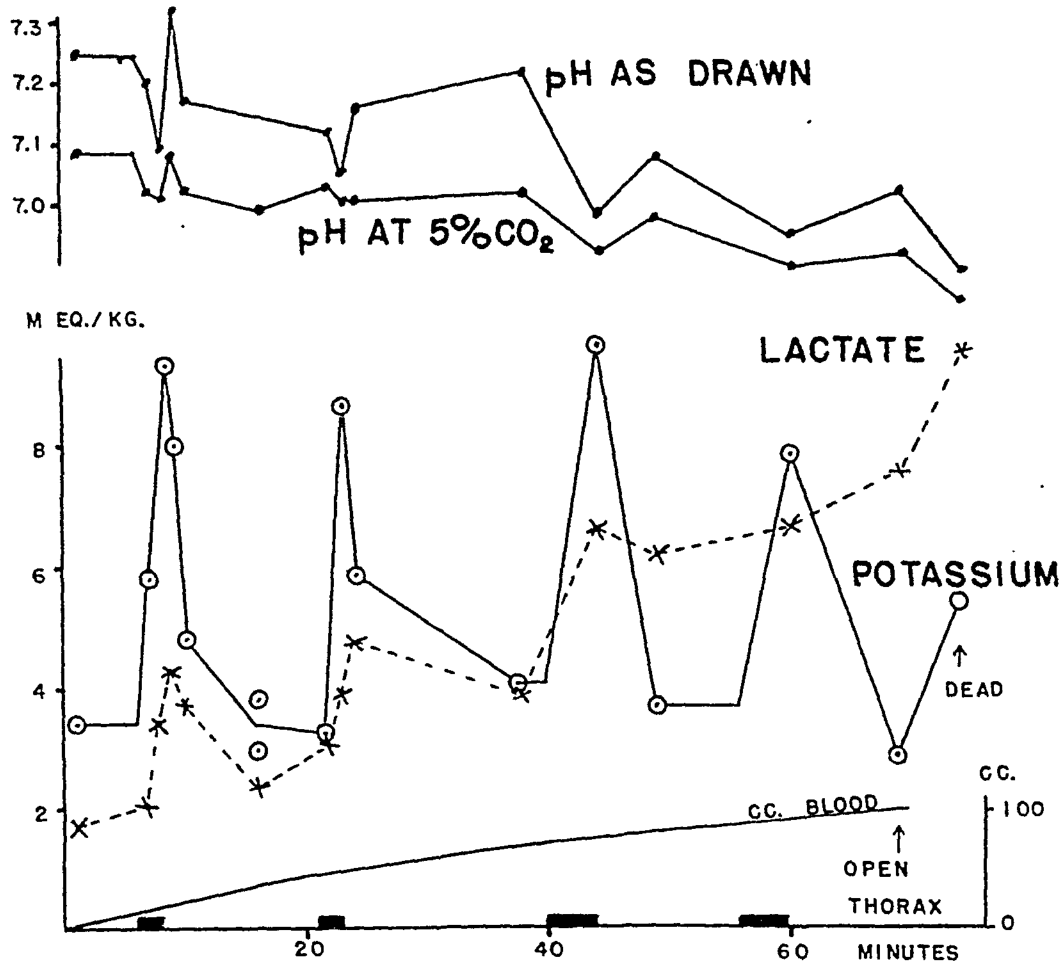


Fig. 1. Effects of four periods of asphyxia produced by clamping the trachea. Two periods lasting two minutes and two at four minutes. Artificial respiration was given at the end of each period until spontaneous breathing was resumed. Cat weight, 3.4 kgm., male. Received 2.2 cc. of dial intraperitoneally. Hematocrit dropped from 35 per cent at the beginning to 23 per cent at the end. Lactic acid refers to whole blood and potassium to plasma. Last sample from the heart; all others from the carotid artery.

acid than of K probably because it can disappear by metabolism as well as diffusion. The potassium never returned to the pre-injection level and indeed it showed a secondary rise after 20 minutes as described by Wilde (1939) while lactic acid finally went lower than its original level. Two minutes after injection, however, the increase in lactic acid calculated for plasma was actually 10 per cent greater than the increase in K and the volumes of distribution of K and lactic acid respectively were only 11.2 and 10.4 per cent of the body weight. In this time, therefore, only small but nearly equal amounts of K and lactate had left the plasma. The increases in concentration in figure 1 occur within a period of the order of 2 minutes and the K and lactate curves run parallel.

same blood after equilibration with 5 per cent CO_2 and 95 per cent oxygen showed smaller or irregular changes usually in the direction of increased acidity. We have records of another experiment identical with that shown in figure 1 in all essential particulars. It would not appear from these experiments that the K in excess of lactic acid could have entered the blood as KOH without any other changes.

It is possible that the potassium came into the blood in order to compensate for the increase in fixed acid resulting from the anaerobic metabolism, but the evidence for such an effect is not impressive. Somogyi (1940) reports an insignificant rise of K after injection of sodium lactate. In one cat we injected 6 cc. of 0.1 M HCl into the artery and found no change in plasma potassium 2 minutes later and only an insignificant increase after 6 minutes just before the death of the cat. In perfusing the legs of frogs lactic acid (half neutralized with NaOH) was added to the perfusate without causing a perceptible increase in the venous potassium. (Before lactic acid (0.005N) 3.34 and 3.81 m.eq. of K per liter; after lactic acid 3.66, 3.62, 4.51 and 3.97 m.eq. of K per liter in successive 2–6 minute periods.) Moreover, according to earlier experiments an increase of an easily penetrating acid like carbonic acid or lactic acid would be expected to cause a migration of potassium in the reverse direction, i.e., from the plasma to the tissues (Fenn and Cobb, 1935).

It is not likely that the reverse is true, i.e., that the lactic acid increased in the blood as a response to the increase in potassium because in two frog perfusion experiments we have injected KCl without finding significant changes in the lactic acid concentration of the blood. It is probable, therefore, that the potassium comes out in asphyxia as a consequence of the sympathetic stimulation and the action of adrenalin and that the lactic acid is the sign of a shift from an aerobic to an anaerobic type of metabolism.

Hemorrhage. In the experiment of figure 1 it can be seen that the lactic acid level steadily increases during the experiment. This may be taken to indicate incomplete recovery from the asphyxial periods; it may also be the result of the hemorrhage which was of considerable magnitude as indicated by the lower graph showing the amounts of blood withdrawn during the experiment. To study further the importance of hemorrhage in this effect some experiments were tried in which cats were subjected to acute hemorrhage by the withdrawal of successive large blood samples for analysis. A typical experiment of this sort is shown in figure 2. Both lactic acid and potassium increase during the bleeding and the pH of the arterial blood as drawn under oil and also after equilibration with 5 per cent CO_2 and 95 per cent oxygen showed steadily decreasing values. An interesting and characteristic variation from this tendency to acidity is seen in the temporary increase in alkalinity of the arterial blood after the

fourth blood sample. This is probably due to the over ventilation observed during the experiment because it does not appear at constant carbon dioxide tension.

Table 1 shows figures obtained in three cats subjected to hemorrhages of this sort. The last three columns give the changes in plasma potassium, whole blood lactic acid, and arterial pH after equilibration at 5 per cent CO_2 and as drawn under oil. Since the change in the concentration of

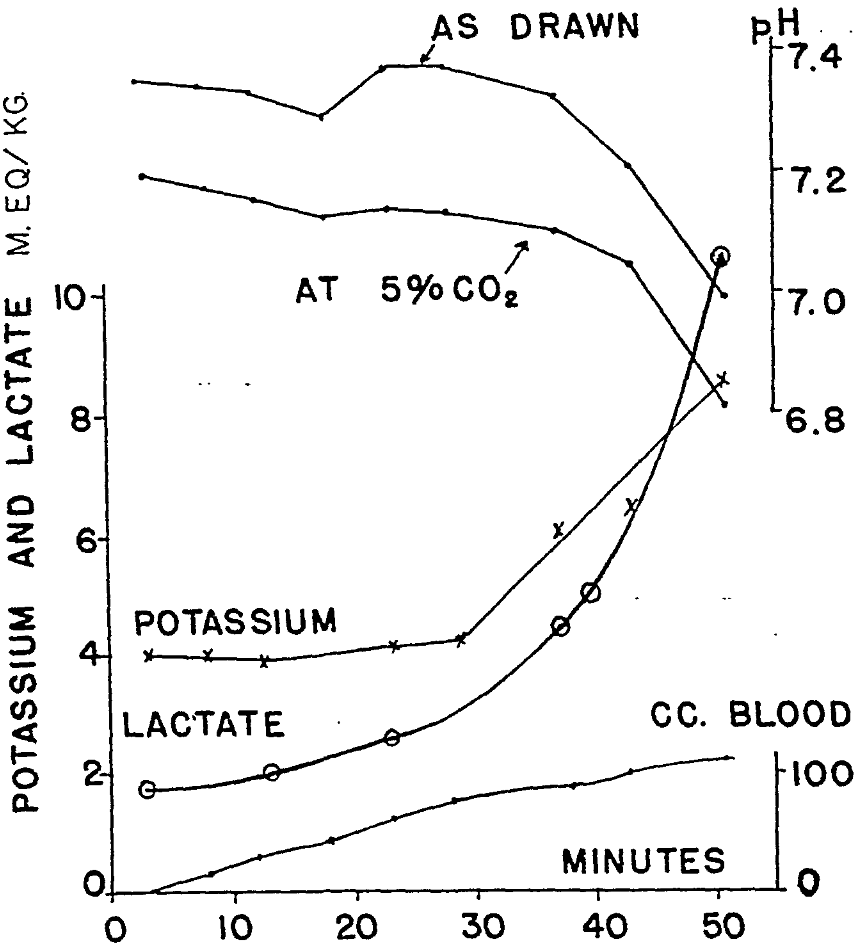


Fig. 2. Effect of hemorrhage in a cat, male, 2.9 kgm. after 1.9 cc. of dial. Last blood sample taken from the heart; others from the carotid artery.

lactic acid in the plasma would be even larger (about 20 per cent) than that in whole blood, it is evident that the number of equivalents of potassium which entered the blood is far less than the number of lactic acid equivalents. It is possible, therefore, that all the potassium came out as lactate but that not sufficient potassium could be mobilized to neutralize all the lactic acid. The data do not prove, however, any necessary connection between the two substances.

It was shown by Johnston and Wilson (1929) that in hemorrhage the

decrease in the alkali reserve was greater than could be accounted for by lactic acid alone. The simultaneous increase in potassium was not taken into consideration by these authors and would make it still more difficult to account for the decrease in alkali reserve. To one sample of cat's blood we added varying known amounts of lactic acid at a constant CO_2 tension of 5 per cent and measured the resulting pH. We found that an addition of 10.3 m.eq. of lactic acid per liter of blood, the average increase observed in the experiments of table 1, caused a decrease of pH of 0.34 from 7.40 to 7.06. While exact comparison with the Δ pH observed in the experiments of table 1 is not strictly justified because of variations in buffering of different bloods, nevertheless it is striking that this figure is so close to the Δ pH at 5 per cent CO_2 actually observed, i.e., 0.36. On this

TABLE 1

Electrolyte changes due to hemorrhage

NUMBER	WEIGHT OF CAT	BLOOD	TIME BETWEEN SAMPLES	INITIAL SAMPLE				CHANGE DUE TO HEMORRHAGE			
				Arterial pH		K	Lactate	Arterial pH		K	Lactate
				as drawn	at 5% CO_2			as drawn	at 5% CO_2		
	kgm.	*cc.*	*min.*			*m.eq./l.*	*m.eq./l.*			*m.eq./l.*	*m.eq./l.*
1	3.4	156	53	7.40	7.25	3.92	1.32	−0.18	−0.52	+3.39	+11.43
2	3.8	98	28	7.72	7.37	4.80	1.63	−0.04	−0.18	+2.70	+10.70
3	2.9	123	54	7.36	7.20	4.09	1.82	−0.37	−0.38	+4.51	+8.78
Average									−0.36	+3.53	+10.30

Cats anesthetized with dial. Samples taken at intervals from carotid artery. Data from first and last samples only are given. After the last sample recorded in the table second cat gave 70 more cc. of blood before death 24 minutes later. The Δ pH at the end was −0.62 as drawn and −0.46 at 5 per cent CO_2. Potassium is calculated per liter of plasma and lactic acid per liter of whole blood.

basis the change in pH is explained by the lactic acid and the change in potassium must be ascribed to something else, perhaps an exchange with sodium.

Adrenalin. The results of six injections of adrenalin in three cats are shown in table 2. Blood samples were taken a few minutes before and one minute after adrenalin in each case. As would be expected from the literature (d'Silva, 1934 and others) there was a marked increase in plasma potassium in each case which is on the average 24 times as great as the small increase in lactic acid which appeared in at least two of the cats. The increase in lactic acid is of the same order of magnitude as that reported by Griffith, Lockwood and Emery (1939). At the same time there was in all cases an increase in corpuscular volume probably due to contraction of the spleen, and a decrease in both Na and Cl. The latter should not

be stressed too much since the changes are small on a percentage basis, and they were followed in only 3 of the 6 injections; but they appear to be consistent and they make it difficult to maintain the theory that after adrenalin the potassium comes out merely as a result of acid-base changes. Certainly in the case of adrenalin the mobilization of the potassium is independent of the lactic acid.

On the theory that small increases in lactic acid observed might have been the result of the high potassium, we injected KCl in experiments 1 and 2 of table 2 between the two injections of adrenalin. The rise in

TABLE 2

Plasma electrolyte changes after adrenalin

NUMBER	AMOUNT INJECTED	WEIGHT OF CAT	BEFORE ADRENALIN					CHANGE AFTER ADRENALIN				
			K	Lactate	Na	Cl	Cell volume	K	Lactate	Na	Cl	Cell volume
	mgm.	*kgm.*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*per cent*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*m.eq./l.*	*per cent*
1a	0.04	3.3♂	4.55	2.15	149.3	120.5	32.0	+3.55	+0.27	−0.7	−2.5	+9.0
1b	0.05		3.74	2.14	151.5	118.5	35.2	+5.20	+0.53	−2.1	−1.8	+5.2
2a	0.05	3.4♀	5.48	2.73			40.3	+5.02	+0.11			+2.5
2b	0.04		5.70	3.60	132.1	117.5	34.9	+3.34	+0.26	−3.3	−7.4	+1.0
3a	0.03	2.6♂	5.60	0.67			46.0	+5.35	+0.08			+9.5
3b	0.03		3.77	0.94			51.0	+6.43	−0.07			−1.0

All three cats were anesthetized with dial. Arterial blood samples (5 ml.) were taken in a syringe containing in the needle 0.14 cc. of a solution of 2.1 per cent Na oxalate and 3.9 per cent NH_4 oxalate. Lactic acid was analyzed in whole blood; Na, K and Cl in plasma. Adrenalin was injected in about 20 sec. intravenously in about 1 cc. of 0.85 per cent NaCl. Samples were taken a few minutes before and 1 minute after adrenalin. At least 45 minutes were allowed between the first and second injection. In experiments 1 and 2 an injection of 5 cc. of 0.2 M KCl was given slowly over a period of 1 minute between the two adrenalin injections. The resulting changes in plasma K were typical and are not reported in detail. The lactic acid changes were insignificant.

plasma potassium concentration which was observed was at least as great as that resulting from adrenalin injection but the changes in lactic acid were quite insignificant.

Further details of these experiments are illustrated in figure 3 where the data of experiment 1 (table 2) are plotted.

Muscular activity. It is well known that in muscular activity there is a loss of both potassium and lactic acid (Fenn and Cobb, 1936). This might suggest an escape of potassium as potassium lactate, but this hypothesis is rendered difficult by the finding that the loss of potassium is accompanied by an almost equivalent gain of sodium. These experiments, however, covered a period of about a half-hour so that it is still possible

that the loss of potassium and the gain of sodium do not occur simultaneously. In that case a loss of potassium with lactate would be possible.

To obtain further evidence on this question a series of experiments was undertaken with cat muscle in which the venous blood from the muscles was collected before, during and after stimulation and analyzed for both lactic acid and potassium. The preparation used was similar to that described by Fenn, Wilde, Boak and Koenemann (1939). A strong ligature was passed around the thigh underneath the femoral artery and vein and the sciatic nerve. The venous cannula was inserted into the saphenous vein close to the femoral and pointing toward the heart so that

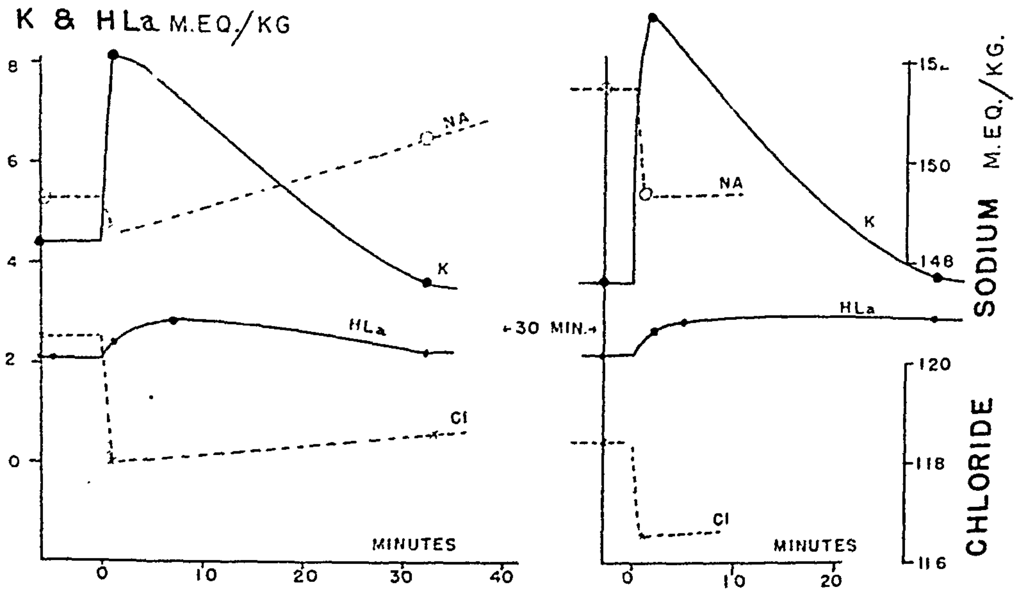


Fig. 3. Effect of two adrenalin injections in a cat. Abscissae, time in minutes after injection. Ordinates: concentrations of K, Na and Cl in plasma and of lactic acid (HLa) in whole blood in milliequivalents per liter. Data from experiment 1, table 2. It is assumed that the concentrations observed before injection did not change until adrenalin was injected.

clamping of the femoral would divert all the blood into the cannula. Clotting was prevented by heparin. When a sample was not needed the blood could be allowed to return to the heart in the usual way by removing the clamp from the femoral vein. The ligature around the thigh gave assurance that all the return blood came through the cannula. Samples of whole blood were used for lactic acid analyses and samples of plasma for potassium.

During the past two years we have done a number of these experiments and have had no difficulty in demonstrating a marked increase in the concentration of potassium in the venous blood which begins apparently

simultaneously with the onset of contraction. Since our experiments were completed a paper by Wood, Collins and Moe (1940) has appeared in which similar experiments were performed on a dog muscle perfused by a heart lung preparation. Our results are similar in general to those reported in the dog with the exception that we have never been able to observe during recovery that the potassium returned to the muscle. In our experiments the potassium level gradually fell in the venous blood to the pre-stimulation level but did not go below that level. Such a return of potassium to the muscle is of course to be expected and our failure to observe it suggests either that recovery is more gradual in the cat than in the dog or that our preparation was not in a sufficiently normal condition to obtain good recovery. It must be admitted that the contractions which we have obtained with this preparation have not seemed to be as well maintained in general as would be expected from our experience with muscles of similar cats in which no attempt to collect the venous outflow had been made. Nevertheless the contractions were initially strong, and there is no reason to suppose that the electrolyte changes, during contraction at least, were not normal.

The changes of potassium and lactic acid in the venous blood for five different experiments are plotted in figure 4. All the blood from a few minutes before the onset of contraction until a few minutes afterwards was collected and analyzed in successive samples. The graphs show that the potassium increases rapidly beginning with the first 10 seconds after stimulation while the lactic acid rises more slowly after an initial lag or even an initial fall slightly below normal. This finding seems to be characteristic of all our experiments. It is striking that the increase in concentration of lactic acid is usually higher than that of the potassium and reaches its maximum only after the potassium level has begun to fall.

These results make it difficult to believe that the potassium comes out into the blood as potassium lactate. The reason for the slower appearance of the lactic acid may, of course, be merely a matter of slower diffusion or of a lower permeability of the membranes to lactic acid. Whatever the reason may be, the fact is nevertheless evident that potassium enters the blood for the most part unaccompanied by lactic acid. It appears likely, therefore, that it enters by exchanging with plasma sodium but the simultaneity of these two processes has not been demonstrated.

Muscular activity in frog perfusions. A further opportunity for studying the potassium loss from muscles in relation to the lactic acid was offered by some experiments in which the hind legs of frogs were perfused through the abdominal aorta. The perfusate was collected by a cannula in the abdominal vein, the renal portal veins being tied off. As a perfusion mixture we followed Saslow (1938) in using a 10 per cent suspension of washed beef red cells in frog Ringer's solution containing 3 per cent acacia.

Further details of this method have been described by Fenn, Koenemann and Sheridan (1940).

If the arterial potassium concentration is high the muscles remove potassium from the blood; if the arterial potassium is low the muscles give up potassium to the blood. This result is a confirmation of the finding of Mond and Netter (1930). The result is illustrated in part by

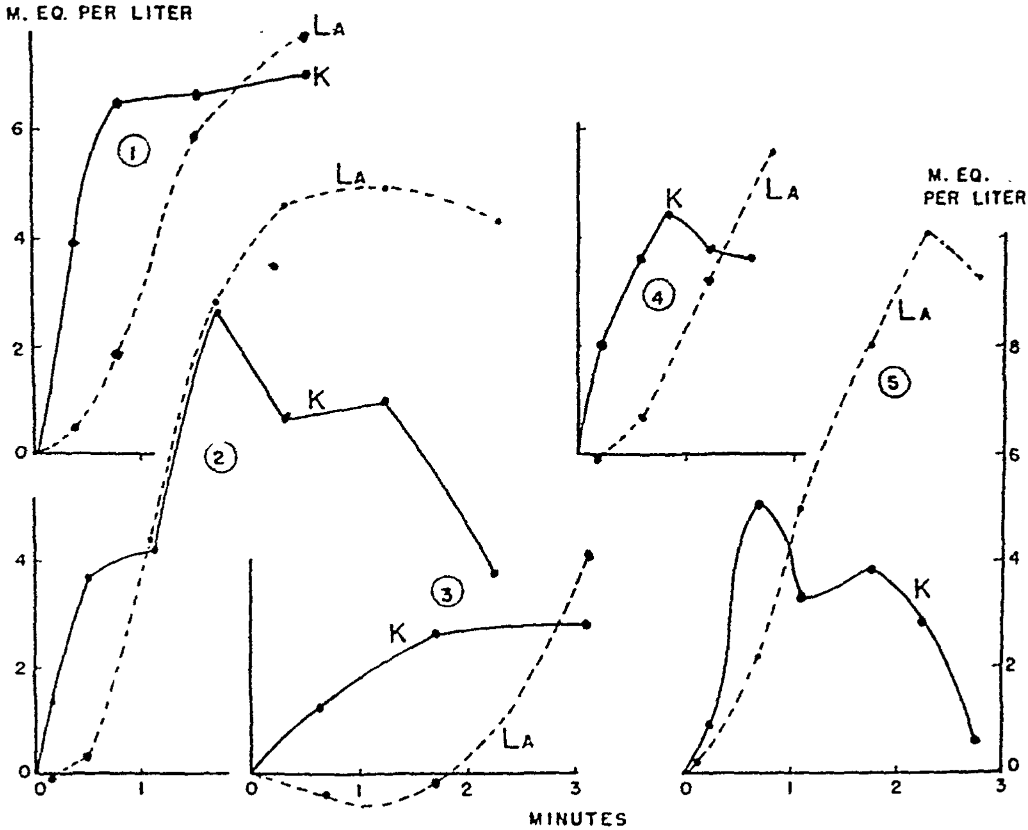


Fig. 4. Graphs of five experiments on cats showing the changes in the potassium and lactic acid concentrations in the venous blood from muscles as a result of electrical stimulation through the sciatic nerve. Dotted lines, lactic acid in whole blood; solid lines, potassium in plasma. Abscissae: time in minutes after the beginning of stimulation. Stimulation in the five experiments: (1) 2.6 min. at 15 per sec., (2) 3 min. at 15 per sec., (3) 4½ min. at 18 per sec.; (4) 3 min. at 42 per min.; (5) 2¼ min. In no. 2 stimulation began 2 min. after the injection of 50 mgm. of iodoacetic acid, and the cat was dead 10 min. later.

figure 5 which represents an experiment in which the potassium content of the arterial blood was suddenly increased for a period and then returned to the original low level. The level of the potassium in the venous blood increases, however, relatively little. Another similar experiment gave an identical result.

The absorption of such large amounts of potassium from the blood afforded an opportunity to investigate the method of uptake of the potas-

sium. For this purpose the pH of the arterial and venous blood was followed both before and after equilibration with 5 per cent CO_2 and 95 per cent oxygen (see fig. 5). In spite of the large amounts of potassium which were being removed from the blood the pH change at 5 per cent CO_2 was negligible. This proves that the potassium did not leave the blood as KOH or in exchange for H ions because titration of the actual perfusion mixture used with known amounts of KOH showed that the buffering capacity was such that the removal of 6.8 m. mols. of K per kgm. from the blood as KOH should have decreased the pH by two units (i.e., from

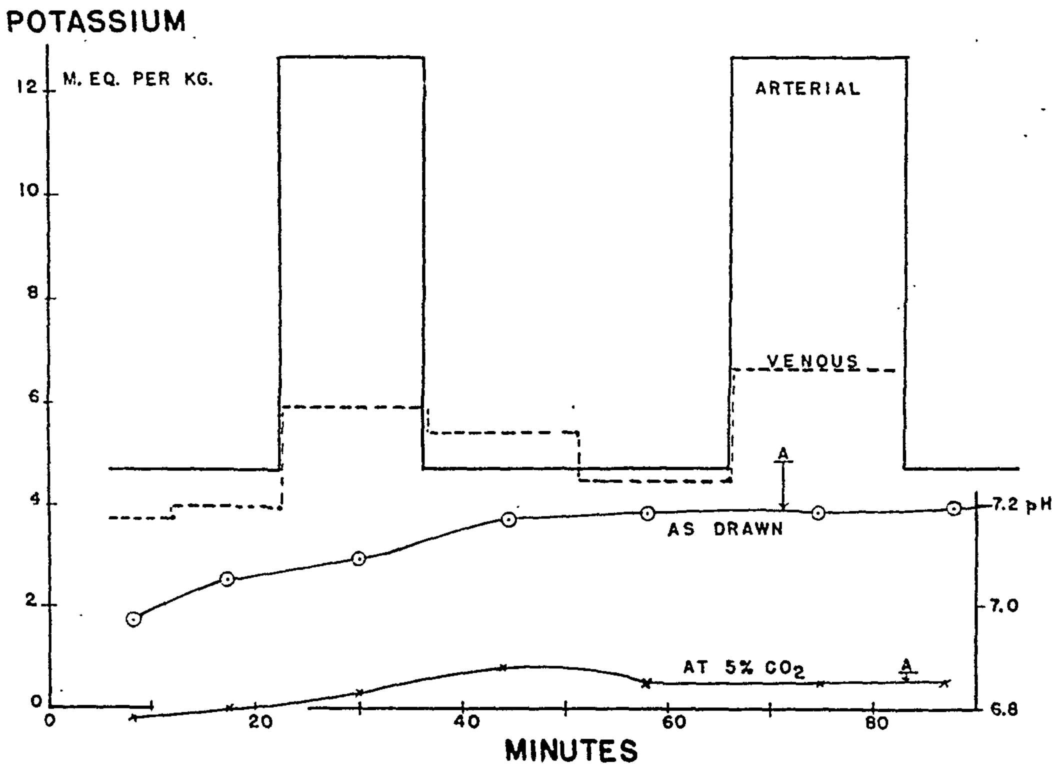


Fig. 5. Effect of varying arterial potassium on the venous potassium concentration in a frog perfusion. The venous pH both as drawn under oil and after being re-equilibrated at 5 per cent CO_2 and 95 per cent O_2 are also plotted. Corresponding arterial pH values are indicated by levels marked *A*.

7.3 to 5.3). The gradual rise of the pH of the venous blood before equilibration (i.e., as drawn) and its approach to the arterial value indicates progressively less acidifying effect due to CO_2 from the tissues and probably means a more rapid flow of blood through smaller fractions of the capillary bed. The total flow decreased from 0.58 cc. per minute at the beginning to 0.15 cc. per minute at the end in spite of an increase of pressure from 21 to 31 cm. of water at 40 minutes on the graph. Increase of K in the arterial blood did not change the arterial pH which was 7.28 in equilibrium with pure oxygen (at either high or low potassium) and 6.87 when equi-

librated with 5 per cent CO_2 and 95 per cent oxygen. These values are indicated on the graphs in figure 5. The slightly lower pH of the venous blood at 5 per cent CO_2 as compared to the arterial probably indicates the addition of lactic acid from the muscles.

Curare. With the same frog perfusion preparation it was possible to test whether the loss of potassium resulting from muscular activity is the result of the contraction or the result of excitation at the neuro-muscular junction. This was done by stimulating after poisoning with curare. After the addition of curare the perfusion was continued until there was no visible response to a single shock. Then the nerve was stimulated with a tetanic current. Analysis of the venous perfusate showed no detect-

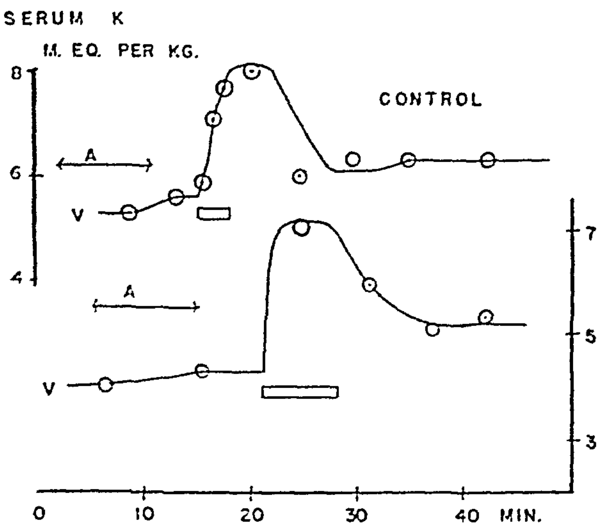


Fig. 6

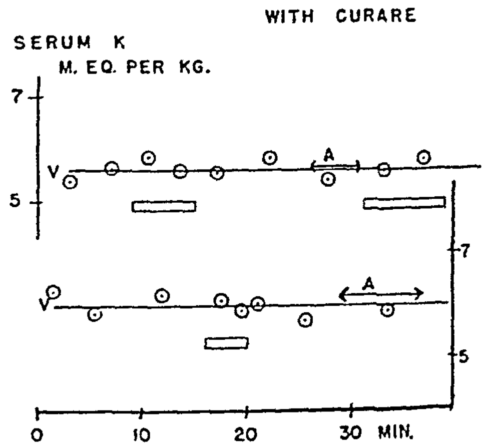


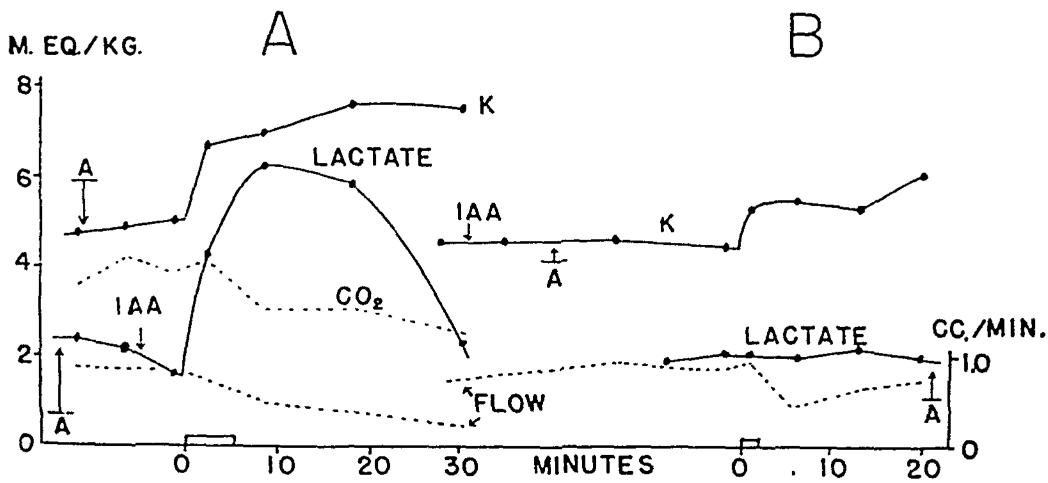
Fig. 7

Figs. 6 and 7. Effects of stimulation on the venous serum potassium content of perfused frog muscles. Figure 6, normal muscles; figure 7, similar muscles poisoned with curare. Blocks indicate periods of stimulation. Points are plotted at the middle of the interval used for the collection of the sample. Corresponding arterial values are indicated by *A*.

able increase of potassium. This fact is illustrated in figure 7, and figure 6 gives 2 control experiments without curare for comparison. Two other unreported experiments with curare gave identical results. Since these experiments were completed, a similar result in the dog has been published by Wood, Collins and Moe (1940). These experiments cast some doubt upon the data of Reginster (1938) showing an increase in the diffusible K after stimulation even when contraction is prevented by curare.

Iodoacetic acid. As a further more crucial test for the independence of potassium and lactic acid in muscular contraction we have studied the liberation of these substances from stimulated perfused frog muscles with and without the addition of iodoacetic acid. The results of two such

experiments are shown in figure 8 A and B. In the former the iodoacetic acid was introduced only 3 minutes before stimulation and did not act for a sufficient time to prevent the formation of lactic acid. Nevertheless the muscles went into rigor after 2 to 3 minutes of stimulation. The potassium in the venous blood remained at a high level, indicating a continued loss from the muscles during rigor. In the experiment of figure 8 B the iodoacetic acid was introduced 30 minutes before stimulation. This resulted in an equally prompt onset of rigor, a smaller contraction, a smaller but continued loss of potassium and no significant increase of lactic acid. Nevertheless this experiment, like others similar to it, indicated that a loss of potassium in contraction can occur without a corresponding loss of lactic acid. It might perhaps be argued, however, that



Figs. 8 A and B. The effects of stimulation of the sciatic nerve of a perfused frog on the lactic acid and potassium in the venous blood, A, 3 min. and B, 30 min. after iodoacetic acid (indicated by arrows). Stimulation periods indicated below by blocks. Arterial levels of K and lactate indicated by A. Abscissae, time from the beginning of stimulation. K in m.eq. per liter of plasma and CO_2 and lactate in m.eq. per liter of whole venous "blood."

the loss of K which is observed is the result of the rigor rather than the result of the contraction per se.

In figure 8 A values of venous CO_2 content are included which show a steady decrease. This would suggest an increased flow but a decrease in flow was observed. Since the arterial perfusate was equilibrated with pure oxygen practically all the CO_2 in the venous perfusate must have been derived from tissue metabolism. It must be concluded, therefore, that the diminished flow indicates a stagnation in these capillaries where the flow was initially least and which therefore contributed most to the CO_2 content of the blood.

A summary of our 15 experiments with iodoacetic acid is obtained from table 3 in which the corresponding changes in potassium and lactic acid are tabulated. For comparison five control experiments without iodo-

acetic acid are included and the experiments with iodoacetic acid are divided into two groups according to the time allowed between the introduction of the poison and the beginning of stimulation. Figures are given in the table for the changes in concentration of lactic acid and potassium which were observed in the first period after stimulation and also the maximum changes which were observed in any later period after stimulation. With only two to twenty minutes for the poison to act there was no appreciable effect but with periods longer than 20 minutes the change in potassium concentration was slightly less than normal, probably due to the smaller contraction, and the change in lactic acid was diminished by an even greater amount. The probable e s of the mean would indicate that the decrease in lactic acid was large enough to be significant. This leads to the conclusion that nearly normal amounts of potassium

TABLE 3

Summary of experiments showing the effect of iodoacetic acid on the average increase of potassium and lactic acid in venous blood due to stimulation

	TIME AFTER IAA	NUMBER OF EXPERIMENTS		Δ K		Δ LACTATE
	min.			*m.eq./l.*		*m.eq./l.*
Control	0	5	a	1.57 ±0.20	a	0.97 ±0.31
			b	1.70 ±0.17	b	1.95 ±0.47
IAA	2–17	5	a	1.46 ±0.32	a	2.03 ±0.67
			b	2.85 ±0.71	b	4.97 ±1.12
IAA	20–63	10	a	0.81 ±0.15	a	0.10 ±0.05
			b	1.14 ±0.14	b	0.18 ±0.06

a = calculated from first period and b = calculated from maximum value in any period following stimulation. Lactic acid is calculated for whole blood and potassium for plasma. The errors given are the probable errors of the means.

may be liberated in muscular contraction without appreciable amounts of lactic acid.

We have also tried stimulating a cat muscle after injection of iodoacetic acid, but have not been able to obtain results differing appreciably from the normal without killing the animal (cf. no. 2, fig. 4). In the course of these attempts we have observed in two experiments (after injection of IAA but without stimulation of muscles) a sudden rise in arterial potassium from 5 to about 10 m.eq. per liter and a subsequent fall toward normal as death approached. In a normal cat there was no corresponding formation of lactic acid but in one cat previously injected with potassium lactate the administration of iodoacetic acid also caused a rise in lactic acid. The meaning of these observations requires further investigation.

Discussion. These experiments have shown that potassium is liberated

into the blood often but not always in company with lactic acid. It could hardly be expected that any one substance could account for all the movements of potassium. All the acids and bases should be considered together. The relative values of the changes in potassium and lactic acid in the different conditions are nevertheless of some interest. In asphyxia and after adrenalin the potassium change is at least twice as great as the change of lactic acid. After hemorrhage the reverse is true. The effect of asphyxia is possibly due to the sympathetic stimulation and liberation of adrenalin which it causes while hemorrhage involves other factors concerned with the loss of blood volume.

In muscular activity the potassium and lactic acid increments are more nearly equal but they have quite different time courses so that they cannot be completely interdependent. The effect of iodoacetic acid helps to dissociate these two substances by decreasing the lactic acid more than the potassium. This evidence is not altogether convincing, however, because it is difficult to be sure that the liberation of potassium in this case is not due to the onset of rigor. It might perhaps be possible to stimulate at such a slow rate that no lactic acid was formed in which case there might still be a perceptible loss of potassium. Although there may still be some connection between potassium and lactic acid insofar as their *liberation* in the muscle fiber is concerned, it is certain that potassium may *appear* in the blood without equivalent amounts of lactic acid.

We have tried various experiments to learn just how potassium enters the blood but so far we are only able to rule out the suggestion that it enters (or leaves) as KOH. The pH at constant CO_2 tension does not change enough to permit this explanation. In two experiments on cats we have tried to settle the question by analyzing arterial and venous blood for Na, Cl, H_2O and HCO_3 in addition to K and lactate before, during and after stimulation. The results remain difficult to interpret, partly because of the small percentage changes in Na and Cl, which are therefore subject to large errors, and partly because of the difficulty or impossibility of knowing for certain just how much venous blood corresponds to how much arterial blood. The conclusions vary according to the basis which is chosen for comparison, whether it be water, solids or red cells, and no one basis is entirely above reproach. In general the water content goes down and the Na, Cl and HCO_3 go up and even on a dry basis they show changes as large or larger than the changes in potassium. It may be, therefore, that the changes in K and lactate are only the most easily detectable but not the most important quantitatively of the acid-base changes which occur in contraction.

SUMMARY

1. In progressive acute hemorrhage in cats the lactic acid increases in the blood more than the potassium (in equivalents) while in acute asphyxia

from clamping the trachea, and after the injection of adrenalin, the increment of potassium is more than twice the increment in lactic acid.

2. In muscular contraction in cats the increments of potassium and lactic acid are more or less equal (or the lactic acid increment is larger), but the rise in lactic acid begins later and reaches its maximum later than does the rise in potassium. In cats the increase in potassium becomes perceptible in a sample of venous blood collected during the first 10 seconds after stimulation begins.

3. In frog perfusions curare completely abolishes the rise in both potassium and lactic acid; iodoacetic acid after acting for a sufficient length of time abolishes or much diminishes the increment of lactic acid due to stimulation without abolishing (although somewhat decreasing) the increment in potassium.

4. Measurements of pH show that potassium does not enter or leave the blood to any extent as KOH. It is also evident that it can enter or leave without equivalent amounts of lactic acid.

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EFFECT OF INSULIN ON NERVE ACTIVITY

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Procedures have previously been employed in this laboratory to determine the action of the convulsant, strychnine, on the nervous system (Heinbecker and Bartley, 1939). More recently the same method has proven successful with regard to the action of two typical anesthetics, ether and nembutal (Heinbecker and Bartley, 1940). Since insulin produces marked modifications in the overt state of the animal attributable to alteration of nervous activity, the same technique has been applied to the study of the action of this substance, the present paper being a presentation of the findings and their interpretation.

The experiments included the investigation of 1, the threshold, accommodation, conduction rate, and absolutely refractory period, of the nerve fiber; 2, the efferent discharge over the phrenic nerve; 3, the afferent discharge over the vagus nerve of sense organs in the lung; 4, several aspects of cortical behavior; in the latter case, among other things, the change in spontaneous activity; the size of the immediate cortical response to stimulation of the saphenous nerve, and of the retina with light; and, also, the insulin effect on the intrinsic periodicity and facilitation in the cortex were studied.

METHOD. Cats were used for the experiments and generally fasted 24 to 72 hours, and in some cases given preliminary doses of 10 units of insulin (Iletin, Lilly) the day before the experiments, to insure that the final doses of insulin administered intravenously at the time of recording would produce a sufficiently marked insulin effect.

In order to immobilize the locally anesthetized animal while recording from the cortex, tetra-methyl-ammonium-iodide was used. About 20 mgm. per kilo were sufficient to produce a curare-like effect, while the drug produced no observable change in the spontaneous cortical records as observed under ether, and did not seem to reduce the size of the cortical response to sensory stimulation as does ether. The drug, however, resulted in a temporary rise of the blood sugar level, and provision for

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its subsequent reduction to definitely hypoglycemic levels had to be made. Figure 1 reproduces two curves to illustrate the time course of the changes in the blood sugar level, the first when the animal was fasted 24 hours but not given insulin on the day of fasting, and the second when insulin was given on the day of fasting. From the curves it is obvious with the dosages of insulin employed, that an hour or more elapses before the blood sugar level drops to the minimum, which even then does not represent a very low value. With longer fasting and repeated insulin injections marked hypoglycemia resulted.

It was in some cases necessary to begin the experiment during deep insulin depression and work back toward the normal by giving intravenous glucose (15 to 20 cc., 5 to 20 per cent in Tyrode's solution). The effects

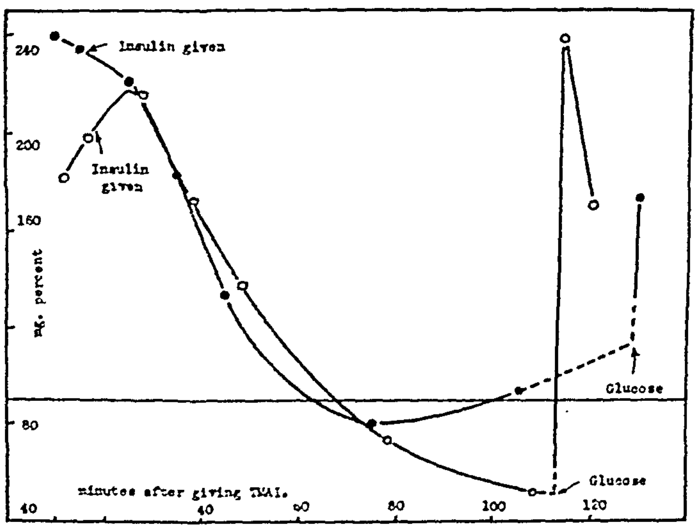

Fig. 1. Herein are two curves showing the blood sugar level following the administration of tetra-methyl-ammonium-iodide. See text for remarks.

of glucose are to be interpreted in light of this. That is to say, if upon its administration the animal was comatose, its effect was to revive it. If the animal was in a convulsive or hyperirritable state, its effect was to quiet it. If given during convulsions, the glucose thus brought the animal back toward the normal state; if given during coma, it also influenced the animal in the same direction, but often into or through the convulsive state.

Results. *The nerve fiber.* To study the effect of insulin on the nerve fiber the animal was first fasted and then given insulin. When the animal was reduced to coma, one saphenous nerve was removed under local anesthesia. In both cases the threshold, accommodation, conduction rate, and absolutely refractory period were determined.

Following the examination of the first nerve, the animal was given glucose, which revived it in a few minutes to the point of becoming spon-

taneously active and very responsive to irritant stimuli. It was then lightly anesthetized with ether and the other saphenous tested in the same manner as the first.

The properties of the first nerve were found to lie within the normal range as indicated by previous studies (Heinbecker, O'Leary and Bishop, 1933). The second nerve was not appreciably different from the first. Figure 2 shows the accommodation curves for both. From the results it can be said that these properties were not modified even when the animal was so deeply depressed that it did not respond to stimuli such as are involved in an operation.

The phrenic discharge. The operative preparation for recording from the phrenic nerve was carried out under local anesthesia and tetra-methyl-ammonium-iodide. The animal was given artificial respiration after cutting the vagi and the efferent discharge over the phrenic studied. To accomplish this the thorax was opened on the right side, the phrenic nerve sectioned above the diaphragm and freed upward for a distance adequate

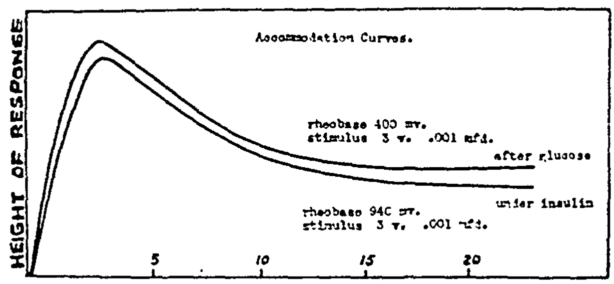

Fig. 2. Accommodation of cat saphenous nerve under deep insulin depression and when revived by glucose.

to permit its placement on the recording electrodes. The lung was kept out of the field by moist packs.

During the excitement state effected by insulin, the amplitude of the discharge was enhanced and prolonged. In addition to this there appeared to be an increase in the discharge frequency of the individual responses in the volley.

Normally, the discharge lasts only the time represented by the usual inspiration (Heinbecker, 1932) and during expiration the nerve is quiet. Our animals were given artificial respiration at a constant rate. The movement of the lungs and chest walls, therefore, set up afferent stimuli which often paced the phrenic discharge. In the border-line convulsive state the discharge, when once set up, sometimes extended for a period comparable to several respiration cycles and thus escaped from its artificial pacing. Not only in this way but in others also synchrony was lost. Under some conditions the phrenic volleys broke up into 3 to 5 bursts during each inspiratory phase of the cycle. At other times the alterna-

tions of bursts and momentary pauses continued through the expiratory phase as well, *without regard to the artificial rhythm and forming a uniformly* spaced train of rapid volleys at a rate corresponding to that which is seen during panting. Vigorous artificial ventilation with air did not eliminate the panting, but it ceased following ventilation with a mixture of 10 per cent CO_2 in 90 per cent O_2.

The characteristic action of glucose was to reduce the prolonged duration and excessive amplitude of the discharge very markedly, sometimes immediately restoring it to normal proportions. It also eliminated the

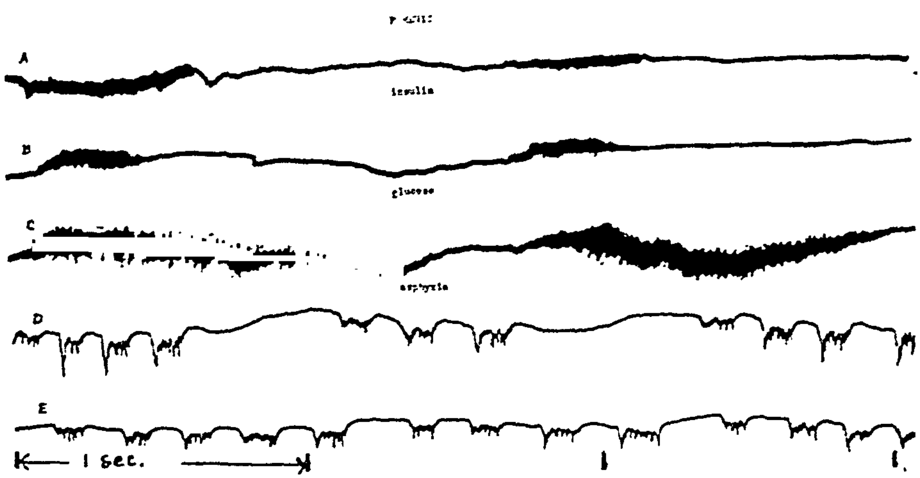

Fig. 3. These records illustrate the kinds of discharge mentioned in the text. A represents the phrenic discharge under a moderate degree of insulinization. When glucose was given with the animal in this state, both the amplitude of the summated discharges in the volley and the duration of the volley were decreased, as shown in B. Line C indicates the effect when artificial respiration is discontinued for about a minute, asphyxia prolonging and increasing the amplitude of the volley. Lines D and E show the phrenic discharge in another animal. In D, the insulinized animal pants during the inspiration phase of the artificial respiration cycle. After the administration of glucose, the panting continues throughout the whole artificial respiration cycle, though the amplitude of the discharges is not so great. This is taken to indicate that insufficient glucose was given to bring the animal all the way back through the hyperirritable stage.

panting rhythm. In the state of coma, however, glucose enhanced the discharge or made it reappear after it had vanished. It should be kept in mind that the enhancement of the response does not follow the administration of glucose to the animal in the normal state, but only when depressed following insulin. (See fig. 3.)

The afferent vagus discharge. Under conditions similar to those used in recording the efferent response over the phrenic nerve, the afferent discharge over the vagus was examined to determine whether during excitement of the central nervous system under insulin administration, the

activity of the peripheral sense organs is also heightened. The nerve was sectioned and freed high in the neck and elevated from the body upon recording electrodes close to the cut end. These electrodes were upon a common carrier provided with a transparent cover, forming a chamber to maintain the nerve in a moist atmosphere (O'Leary, Heinbecker and Bishop, 1934). The lungs are supplied with sensory endings of the vagus nerve which are activated by the expansion of the lungs.

In our experiments, no indication of any increased activity was obtained. From this it is assumed that sense organ responsiveness was not heightened during the convulsive insulin state.

Cortical response—relation of size to stimulus strength. The shape of the response of the sensorimotor cortex changes both in the untreated and in the insulinized animal as the strength of stimulation of a peripheral nerve is increased. In our experiments, the saphenous nerve was sectioned at its distal end and elevated onto the electrodes in the chamber previously referred to. With weak stimuli activating only the A fibers, an early diphasic or triphasic response is evident. With stronger shocks a definite second component, predominantly negative in sign, also appears, and becomes confused with the negative component of the weaker response. This is elicited by the delta fibers in the nerve whose conduction rate is slower. Whereas the onset of the first component followed the beginning of stimulation by about 8 to 15 milliseconds, the peak of the negative wave of the record approaches a value of 45 to 50 milliseconds. With still stronger stimulation, activating the C fibers in the nerve, a much later component also appears. Its latency is about 400 to 500 milliseconds. Due to the way the components overlap it is not easy to detect all increases in size of response. The increase in a given component may appear to diminish one of opposite sign partly concurrent with it (Heinbecker and Bartley, 1940), since the positive phase of the second compensates the negative phase of the first.

In the insulinized animal, the *size* of the immediate response of the sensorimotor cortex to saphenous nerve stimulation continued to increase to a greater degree than normal over the entire range of the saphenous "nerve spectrum." This, of course, did not apply to all states of insulinization, but only to the excitement stage, as judged by other criteria, such as increased muscle tone and lowered threshold for reflex response. It is pertinent to point out that this enhancement of the cortical response was similar to that found under strychninization, but less marked (see fig. 4).

The administration of 5 to 10 cc. of calcium gluconate intravenously greatly reduced and sometimes obliterated this enhancement. This, too, is similar to what happens under strychninization, where calcium is able to prevent or to reduce the severity of convulsions.

Cortical response—flashes of light. The cortical response to a flash of

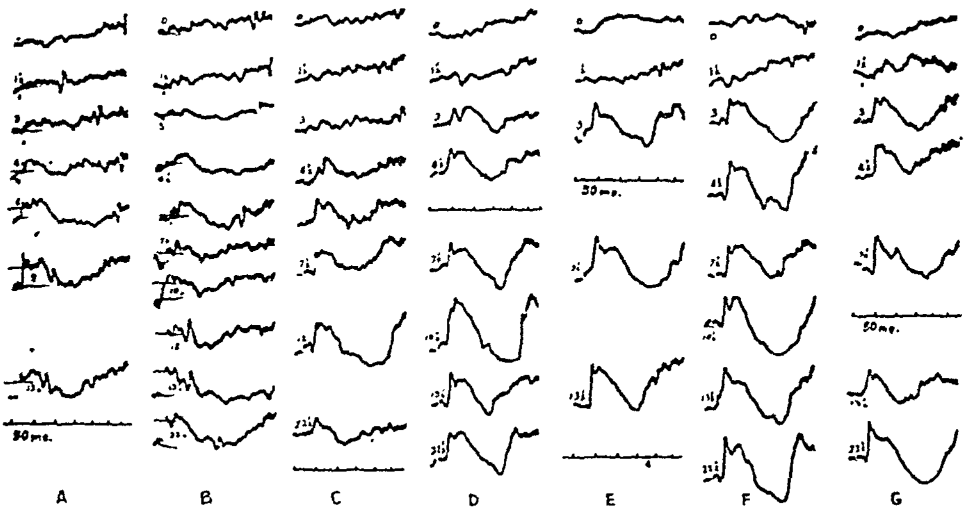

Fig. 4. In this figure the enhancement of the cortical response to electrical stimulation of the saphenous nerve is illustrated by the use of a step-wise series of intensities varying from 1.5 to 22.5 volts, each column of records with the animal in a different state. The stimulus is indicated by a break in the line preceding the response. Column A indicates the outcome with the animal fasted 36 hours, but prior to giving insulin. The threshold stimulus was in the neighborhood of 3 volts, and an increase in the stimulus voltage to 13.5 made little change beyond that of 4.5 volts. In the records the stimulus distortion causes the responses at first glance to be larger than they really are, but closer inspection will reveal their true size and their failure to increase much as stimulus strength is raised. Column B includes records taken 6 minutes after the administration of 10 units of insulin. This period of time made little change from the previous recordings. Column C was recorded 19 minutes after the injection of insulin. By this time the insulin had been able to affect the blood sugar level of the animal. The overt change consisted in hyperirritability and panting. The records show a general increase in the response size, even near threshold, and a greater augmentation of the response as stimulation is made stronger, the effect not stopping at a stimulus strength of 4.5 volts. As was said before, the way in which the components of the response overlap produces a series which does not show a uniform increase in size throughout the range, though the series taken as a whole bears out the conclusion that the components of the responses do increase.

Column D was recorded a couple of minutes after giving about 15 cc. of 15 per cent glucose. The animal had both lost its hypertonicity and ceased panting. Note that the size of the responses has become still greater and the increase over the 4.5 volt level is still more apparent. The glucose evidently changed the animal toward both greater irritability and responsiveness.

Column E was taken about 5 minutes later. At this time the blood vessels had largely recovered from collapse and constriction, typical under insulinization, and which must have lessened the blood supply to the brain and the remainder of the central nervous system and thereby have been responsible in part for some of the symptoms. Twenty minutes after records in column C, column F was recorded, three minutes after a second injection of glucose. The records are even larger than previous ones, and the same increase with stimulus strength still appears. Column G was taken 18 minutes later. The only difference apparent is the dwindling of the relative sizes of responses throughout the whole range from what they had been previously.

light repeated every 4 to 5 seconds was also recorded, with the animal in various stages of insulinization (see fig. 5).

From the consistent results on four animals, it may be said that insulinization can modify the immediate response of the *optic* cortex. It enhances the response during the time the animal exhibits increased muscular tone or is in a border-line convulsive state. Glucose reduces the responsiveness of this cortex promptly, as also does calcium.

Cortical response—paired or repeated stimuli. In the normal animal two stimuli adequate to stimulate only the large A fibers in the saphenous nerve will summate to produce a single cortical response only when they are no more than a few milliseconds apart. Separations greater than about

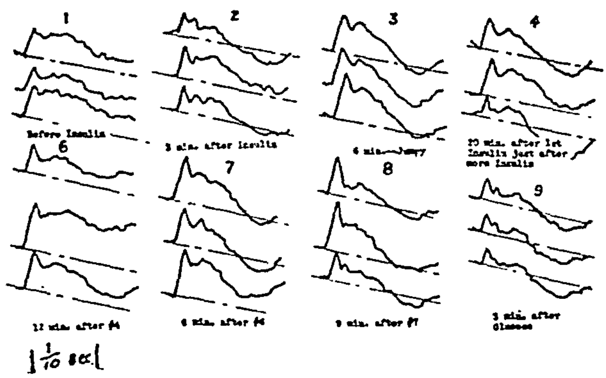

Fig. 5. This figure pictures the response of the optic cortex to retinal stimulation under several conditions: (1) before insulin; (2) 3 minutes after an injection of 8 units of insulin; (3) 6 minutes after the injection. The animal has become hyperirritable and hypertonic, as evidenced by over-response to tapping on the flanks. The cortical response likewise is enhanced; (4) 20 minutes after the first injection and just after a second dose of 4 units: (6, 7, and 8) represent responses during a subsequent period of 27 minutes during which time no further enhancement of response is evident; (9) taken 3 minutes after the injection of 20 cc. of 15 per cent glucose solution, showing a diminution in the size of the response. The onset of stimulation coincides with the beginning of the line.

5 or 6 milliseconds cause the second stimulus to become ineffective (at least under light anesthesia) until the separation is extended to about 30 milliseconds. Beyond this, both shocks are separately responded to, and with further separation the second shock finally elicits a response equal to the first.

After insulin depression has reached a certain point, the stimuli which were originally effective may fail to elicit response when given singly, but may summate when paired or repeated.

In one experiment, in which the animal was fasted and also given insulin the day before, as well as just before recording, the first observation revealed this refractory interval to be 34 milliseconds. Twenty-two minutes later the interval was 80 milliseconds. Fifteen minutes later,

3 cc. of 20 per cent glucose solution was administered intraperitoneally. In 4 minutes the interval had become reduced to 44 milliseconds, and 6 minutes after that the interval had diminished to 31 milliseconds. It is pertinent to note that this same summation phenomenon was observed with animals under strychnine.

Under insulinization the response to the second stimulus never becomes larger than the response to the first. This contrast between the normal and insulinized animal is brought out in figure 6, in which there is a curve for each of the two states. In both cases, it will be noted, there is a first summation period in which the two responses produce a unitary response, larger than either one alone. This is followed by a drop in each curve, representing the onset of the period during which the second stimulus is apparently ineffective. The drop begins later in the insulin curve than

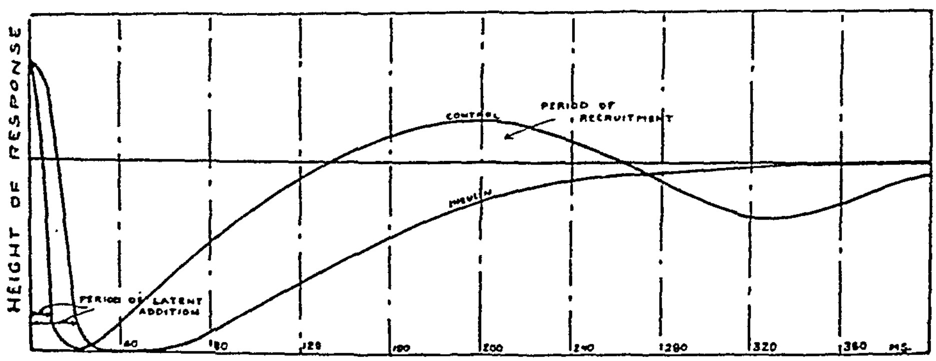

Fig. 6. A diagram showing the relative heights of the response to the second of two stimuli before and after the administration of insulin. Note the periods of latent addition and of recruitment. The latter occurs only under the most favorable conditions. Under insulin it is eliminated, the late period of full effectiveness of the second stimulus is postponed and there is no minimum following the first maximum in the curve.

in the other. After a while both curves rise again. The insulin curve rises later and slopes off to a plateau at about the level of the horizontal line marking the height of a response to a single shock. The other curve rises earlier and extends above the line, indicating a period during which facilitation may occur when the individual shocks of repeated stimulation are separated by intervals represented in the diagram. This phenomenon was pointed out recently by Heinbecker and Bartley (1940). With separations greater than indicated for the maximum second response, a period of diminution sets in, to be replaced still later by a second maximum.

Rhythmicity in the size of the cortical response was first pointed out by Bishop (1933). This phenomenon was brought out very clearly by Bartley (1936) in studying the response of the optic cortex to electric shocks applied to the optic nerve. Most of his animals were under very light ether anesthesia. When dial anesthesia was used the result was similar,

but the period of the excitability rhythm was longer, corresponding to the slowing of alpha rhythm under dial.

Spontaneous cortical activity. Not only are there definite changes in the cortical response to peripheral stimulation, but there are also alterations in spontaneous cortical activity, observable under various degrees of insulinization and following the use of substances which reverse the symptoms. The observations made here are of similar type to those made of the spontaneous activity when the animal was placed under the influence of strychnine, ether, or nembutal.

The effects on both specific and spontaneous responses may be summarized as follows: Glucose and calcium, each may reduce the size of the specific cortical response. If the animal is comatose, glucose restores it to general responsiveness, and restores the much diminished or vanished specific response we are measuring. Calcium does not have this latter effect. It only reduces the response in a hyper-responsive animal (Heinbecker and Bartley, 1939). Calcium increases the frequency of the alpha rhythm in the insulinized animal and diminishes the amplitude of the waves involved when large. Glucose and calcium increase the frequency of the alpha rhythm above the rate found under insulin, and tend to restore toward normal the amplitude of the waves exaggerated by insulin, the original slowing having been similar to that found by Hoagland, Rubin and Cameron (1937).

Comparison of cortical and sub-cortical responses. In animals so deeply comatose from insulin that the spontaneous waves of the cortical record had disappeared, electrodes placed in the basal ganglia of the opposite side still recorded marked activity. In some cases the animal had progressed so far that giving glucose did not to any degree restore the waves. In others, a slight restoration was possible. The retention of a recordable active state in the basal ganglia after the cortex is totally depressed and beyond restoration is not at all surprising, for animals are generally viable when the cortex for any reason fails to give a record. Nevertheless, we have herein a demonstration of one of the differential effects of insulinization.

Discussion. As was already pointed out, our results show that the effect of the hypoglycemic state resulting from insulin administration on the excitation and response processes of the central nervous system of the cat are similar to those already described for strychnine administered intravenously (Heinbecker and Bartley, 1940). The maximum excitatory effects obtainable following insulin action are less pronounced and less prolonged than those possible after strychninization. This follows reasonably from the fact that insulin administration must soon deplete the cells of the central nervous system of the carbohydrate necessary for their function (Holmes and Holmes, 1925). The results, showing that the intravenous administration of glucose will restore to normal within a

few minutes markedly depressed function in the central nervous system, strongly support the contention of other investigators (Himwich and Nahum, 1932) that glucose is the essential material for nerve cell activity. Recovery from a similar degree of depression produced by strychnine is much slower and cannot be effected by glucose administration. Apparently strychnine thwarts the chemical reactions necessary for nerve cell function, whereas hypoglycemia deprives them of essential material without being toxic. Clinical experience indicates, however, that the acidosis associated with prolonged hypoglycemia is also toxic in that recovery from it following glucose administration is a relatively slow process.

The exciting effects of hypoglycemia as well as those of strychninization modify activity in the vasomotor centers in a manner to bring about both arterial and venous constriction in the body generally. We have observed that this results in both ischemia and asphyxia. In the depression period following the excitation the peripheral constriction of arteries and veins is descreased. Cardiac dilatation, especially of the right heart, is evidence of a coincidental weakening of the myocardium. This perpetuates and intensifies the asphyxia with its resulting depression of all cellular activity.

Experiments have been carried out in this laboratory to determine the effects of carbon dioxide and of anoxemia on the function of the peripheral nerves (Heinbecker and Bishop, 1928) and of the central nervous system (Bishop, 1930, unpublished data by the authors). The effect of carbon dioxide (10 per cent in 90 per cent oxygen) is to produce excitatory effects similar to those produced early in hypoglycemia and by mild strychninization. The effect of anoxemia (inhalation of pure nitrogen) is essentially depressant with sometimes a brief early period of excitation if the anoxemia is effected rapidly. According to theory that may be the result of an accumulation of acid metabolites rather than a direct effect of the deprivation of oxygen. These facts are referred to because it is felt that the effects of hypoglycemia and of intravenous strychninization which manifest themselves in the intact animal under our experimental conditions are not simple but represent the end-result of an integration of many factors.

The work in this laboratory has dealt, from time to time, with several aspects of cortical response; namely, its spontaneous features including the alpha rhythm, the effectiveness of the second of two supraliminal peripheral stimuli, and of repeated stimulation, the summation of two sub-liminal peripheral stimuli, and the effect of drugs on these phenomena.

It has become progressively clearer that in such experimentation we are dealing with two groups of cells, one expressed in the immediate specific cortical response to peripheral stimulation, and the other expressed in the alpha rhythm. The second group is spontaneously active and the first is not. Normally the two groups react on each other. A specific response tends to set in motion an alpha train, while the height of a specific response to an isolated stimulus is partially dependent upon where in the alpha

cycle it falls. Strychnine topically applied differentially affects the two, the activity of the spontaneous elements disappearing while certain components of the specific response still may be enhanced. Under strychnine the specific response, though itself enhanced, may no longer set off an alpha train. This is not due simply to synaptic blocking between the response and spontaneous groups but also to an actual depression of the alpha elements inasmuch as alpha waves no longer appear spontaneously. Any repetitiousness that local strychnine produces is manifest in the activity of the non-spontaneous, non-rhythmic elements responsible for the immediate specific response. The effectiveness of repeated stimulation is dependent not only upon the place in the alpha rhythm (spontaneous excitability cycle) each stimulus falls, but also on the spacing of the stimulation and the amount of repetition. This still holds even in the absence of discernible spontaneous alpha waves. Possibly this is indicative of a close similarity in the time course of the excitability processes of the spontaneously and non-spontaneously active elements. Any substance, so far tested, which modifies the rate of the alpha rhythm, whether it is to slow it or accelerate it, produces a like effect on the period which must elapse before a second of two stimuli will produce a measurable immediate cortical response.

A duality of cell groups such as we have evidence for in the cortex, exists in the median nerve cord of the Limulus heart (Heinbecker, 1936). In it there are large pacemaker cells which are spontaneously and rhythmically active. There are also smaller cells which are not spontaneously active but are activated by the pacemaker cells. The integrated outcome of the activity of the pacemaker cells is a slow heart beat of about 20 per minute, each cell being active only a small part of this cycle. The response represents the integration of a progressively spreading activity in a chain of elements. The result of extrinsic excitation of this system depends upon the phase of the cycle at which the stimulus is delivered. A strong stimulus when properly placed elicits a response consisting in the activity of those large and small cells which have recovered from their just previous activity and in the setting up of a new rhythm in definite relation to the stimulus, the old one generally having disappeared. This prototype, inasmuch as the evidence gained from it is more direct, helps to justify our interpretation of the phenomena of the cortex and to clarify what is undoubtedly a more complex situation.

A correlation between the changes effected by hypoglycemia on the elements of the nervous system and the reactions of the body as a whole in this state is of interest. A basis for such excitatory effects as hyperirritability, sweating, trembling and convulsions has been afforded. The mental confusion of depression associated with the convulsive seizure doubtless is an expression of early cortical depression at a time when the lower brain centers and the spinal cord are still hyper-reactive.

SUMMARY

Responses of various parts of the nervous system under insulin were compared with those under standard conditions approaching the normal as nearly as possible, and with the results under strychnine.

The *excitatory* effects following insulin administration are evidenced by a lowering of the threshold for the immediate cortical response to stimulation of the saphenous nerve and an increase in its amplitude and duration. This is associated with an intensification and prolongation of the discharge in certain peripheral axons as is illustrated in the efferent discharge over the phrenic nerve.

Peripheral sense-cells evidently do not participate in the insulin excitatory effect, for no enhancement of the afferent discharge of the vagus nerve was obtained in the hyperexcitable state of the animal. The same lack of excitation is true of nerve fibers.

Depressant action of insulinization is first exhibited in the slowing of nervous processes and later in their diminution and failure. Depression is exhibited in the prolongation of the summation interval (interval of latent addition) and the recovery period of the cortex following peripheral nerve stimulation; by reduction of the cortical response; and by the obliteration of facilitation as measured in our experiments. Depression is seen also in the slowing of the alpha rhythm.

The susceptibility of the different parts of the nervous system to the action of hypoglycemia varies greatly. The cortex may be depressed to extinction at a time when the basal ganglia and the medullary centers still show well integrated activity. Furthermore, the basal ganglia are excited to a greater degree than is the cortex itself. The peripheral synapses, peripheral sense organs and axons are unaffected even just prior to the death of the animal.

The general concepts which have been arrived at through extended work on the nervous system, particularly the cerebral cortex, in this laboratory were discussed in order to orient the findings on insulin of this particular study.

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THE RATE OF GLYCOGENOLYSIS IN THE ISOLATED LIVERS OF SEVERAL SPECIES OF LABORATORY ANIMALS

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It is generally believed that the rate of breakdown of glycogen in the excised liver is extremely rapid. Most investigators have emphasized the necessity for the rapid destruction of glycogenase in the determination of liver glycogen or in its preparation from this source (Bodansky and Fay, 1931). On the other hand even more rapid glycogenolysis may occur in the excised brain tissue. Kerr and Ghantus (1937) found that 80 to 85 per cent of the glycogen is lost from this source within 15 minutes of post-mortem autolysis. In fact Kerr (1938) states that "the interval between excision from the living animal and fixation in alkali should not exceed 10 seconds since glycogenolysis proceeds rapidly (Kerr and Ghantus, 1937)." The glycogen in the abalone muscle on the other hand is extremely resistant to hydrolysis *in vitro* as demonstrated by Petree and Alsberg (1929) who were able to prepare large amounts from the tissues of these animals after long periods of storage. We have repeatedly confirmed these observations in this laboratory.

Cori, Cori and Schmidt (1939) have noted that the diastase content of the blood of rabbits is low compared with such other laboratory animals as the dog, rat and guinea pig. In the perfused liver of the rabbit the rate of glycogenolysis was found to be very slow unless phosphate was added. In the latter case a maximum of 70 per cent disappeared in one hour whereas in control tests the rates varied between 4 and 12 per cent in a similar interval.

In the present investigation, a study has been made of the rate of disappearance of glycogen from the livers of well-fed rats, pigeons, guinea pigs, rabbits, and a dog when this organ was excised and kept at a temperature of 37°C. for various periods of time.

METHODS. In most cases the animals were given large amounts of glucose approximately 12 hours before being sacrificed. Amytal was used as an anesthetic. The liver was divided into several portions, which were weighed in tared centrifuge tubes, and 40 per cent KOH was added immediately to one sample.

The other tubes were kept in an incubator at 37°C. for appropriate periods after which the glycogen remaining was determined. In all cases glycogen was estimated by the procedure of Good, Kramer and Somogyi (1933).

RESULTS. The most satisfactory method for comparison of the rate of glycogenolysis in the various species studied seems to be on the basis

TABLE 1

The liver glycogen in per cent (columns I) and the per cent decrease (columns II) after standing at 37° for various periods after excision from animal

TIME OF INCUBATION	PIGEON		RABBIT		DOG		GUINEA PIG		RAT	
	I	II	I	II	I	II	I	II	I	II
hours										
1	9.51	0	7.51	12.9	4.10	10.5	6.63	16.9	3.18	31.2
1C	9.46		8.63		4.58		7.98		4.62	
	(1)		(3)		(1)		(2)		(6)	
3	7.18	15.0	6.75	21.8	3.60	21.4	3.92	21.3	2.10	55.8
3C	8.45		8.63		4.58		4.98		4.75	
	(2)		(3)		(1)		(2)		(11)	
6	6.92	24.0	6.05	29.9	3.13	31.7	3.71	36.9	1.52	73.6
6C	9.11		8.63		4.58		5.88		5.75	
	(2)		(3)		(1)		(2)		(8)	
12	7.58	36.4	4.33	49.8	1.58	65.5	2.62	62.7	0.84	84.2
12C	11.92		8.63		4.58		7.02		5.33	
	(2)		(3)		(1)		(2)		(7)	
24	6.76	44.7	3.09	65.5	0.62	86.5	1.12	82.5	0.59	89.7
24C	12.22		8.95		4.58		6.40		5.72	
	(2)		(4)		(1)		(4)		(7)	
48	5.14	43.2	3.32	63.7						
48C	9.05		9.14							
	(3)		(2)							
72	2.01	26.1	3.55	64.2						
72C	2.72		9.91							
	(1)		(1)							
96			3.32	66.5						
96C			9.91							
			(1)							

Periods marked C represent the level of glycogen before incubation for the group concerned.

Figures in parentheses represent the number of animals used in each test. There are the same number of control as experimental values in each case.

of the percentage decrease from the original level. This value is calculated as follows: (Control per cent − Per cent after incubation)/Control per cent.

The average per cent decrease of liver glycogen at the various periods investigated as well as the absolute glycogen values are summarized in table 1.

Glycogenolysis in excised liver tissue apparently is not as rapid in any case as is generally supposed. After one hour an average of 31 per cent had disappeared in the rat liver, while only 10 to 16 per cent was lost in the case of the dog, rabbit and guinea pig. No appreciable change was found with the excised pigeon liver at this period. After 12 hours the liver glycogen had reached a low value with the rat liver, although appreciable quantities still remained with the other species. In fact about 75 per cent of glycogen was still found after 72 hours in the pigeon liver and 34 per cent after 96 hours in the case of the rabbit liver.

Although the control levels are considerably higher in most cases in the experiments on pigeons and rabbits, the slower rate of breakdown of glycogen can not be ascribed to that fact. For example, in the one hour period no demonstrable decrease was noted in the case of the pigeon, while the average drop in liver glycogen with the rats was 1.44 per cent. The corresponding lowering for the dog, guinea pig and rabbits was 0.48, 1.35 and 1.12 per cent respectively. Even after the 6 hour incubation period, similar discrepancies were to be noted. Thus the decrease amounted to 2.19, 2.58, 2.17 and 1.45 per cent respectively for the pigeon, rabbit, guinea pig and dog while the corresponding value for the rat was 4.23 per cent. Moreover, an inspection of the individual experiments indicates no definite correlation between the height of the glycogen level at the start of the experiment and the extent of breakdown. After 6 hours the decrease in amount of glycogen in the livers of rats does not exceed that of the other species because it is approaching a blank value. Thus it appears that the rate of glycogenolysis is most rapid in the liver of the rat followed in order by that of the dog, guinea pig, rabbit and pigeon.

SUMMARY

The rate of glycogenolysis in excised liver tissue kept at 37°C. has been found to be much slower than generally believed. The most rapid rate obtains with the rat followed in order by the dog, guinea pig, rabbit and pigeon. In the last case nearly 75 per cent of the original was still found after an incubation period of 72 hours.

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A STUDY OF THE NERVE-FREE SMOOTH MUSCLE OF THE AMNION OF THE CHICK

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The direct effect of physical and chemical agents on muscle tissue can be unequivocally studied by using naturally occurring nerve-free muscle tissue. A number of sources of naturally occurring nerve-free muscle are available. That found in the blood vessels of the human placenta and umbilical cord has been used; but at term such vessels are senile and the premature placenta is not readily available. The aneural embryonic heart has also been used, but graphic records of its activity cannot be made. That found in the amnion of the chick is perhaps most readily available and can be prepared so as to make graphic records.

The structure of the amnion musculature of the chick has been studied rather extensively, more recently by Verzar (1), Pierce (2) and Lewis (3). The absence of nerves has been confirmed by many (1–6). Although the function of the musculature is unknown, it has been suggested (7) that it agitates the amniotic fluid and gently rocks the embryo. *In situ* in the hen's egg, 5 to 14 days after incubation, the amnion musculature has been observed to contract rhythmically at a rate of from 16 to 20 per minute (1), and apparently in the form of waves (2). Epinephrine inhibits the muscle in situ, according to Langley (8). According to Baur (6), isolated strips of the amnion of the goose and chick respond to drugs. This work was undertaken to investigate further some of the physiological and pharmacological properties of this nerve-free smooth muscle and to repeat some of Baur's observations.

EXPERIMENTAL. *Examination for nerves.* Although nervous tissue has never been found in the amnion, a search was made by the methylene-blue technique (9), using the tissue from embryos incubated for from 8 to 16 days. Twenty-four specimens were examined, using a 0.15 per cent solution of the dye in normal saline and incubation at 37°C. A portion of the specimen was examined at periods up to 45 minutes. No nervous tissue was found.

In the remainder of this work, the specimen was always dissected in an isotonic bath at 39°C., using the solution of Van Dyke and Hastings (14) or of Sollmann and Rademackers (12).

Method of recording contractions. The amnion of a chick from a small breed, as used in this work, is a rather delicate structure. In order to record contractions, a lever consisting of a very light paper straw was used. To reduce friction a loop of silk thread served as a fulcrum. Prior to the 10th day the structure was found to be too delicate to produce good graphic records; after the 14th day rhythmicity disappeared. So the specimens used in this study ranged from the 10th to 13th day of incubation, the fertilization time being known.

Solutions used in the bath. It was first thought advisable to use a solution having the chemical composition of the amniotic fluid of the chicken. It was found that the composition, specific gravity, and pH of the amniotic fluid varies considerably after the 9th day of incubation (10, 11). The composition of amniotic fluid, then, is not identical with blood plasma, and, since the salts in bird's blood range from 0.9 to 0.95 per cent, mammalian saline solutions were used in the bath.

Several saline solutions were used in a preliminary study to determine which was the most suitable: Locke's and Tyrode's with their various modifications (12), Sollmann and Rademaekers' (12), Alexander and Hastings' (13), and Van Dyke and Hastings' (14). The Alexander-Hastings solution depressed and abolished rhythmicity (15), and was not used. We did not try a tissue culture medium (3). The Van Dyke-Hastings solution hereafter called D-H solution, was the most satisfactory for the preservation of rhythmicity, it being aerated with 5 per cent CO_2 and 95 per cent oxygen. The Sollmann-Rademaekers solution, hereafter called S-R solution, was also satisfactory, it being aerated with air by the usual method. The pH of the solutions was shown to be constant throughout the duration of the experiments. The pH of the S-R solution was 7.8, of the D-H solution, 7.4. The volume of fluid in the bath was 240 cc. and the isolated strip was suspended deeply so that the composition of the fluid could be altered without mechanically disturbing the strip.

The effect of temperature. The optimal range of temperature for rhythmicity was found to lie between 38° and 42°C. Six preparations were studied to determine more carefully the optimum temperature. In these preparations it was found to be 41°C., which is approximately the temperature of a bird. The bath was maintained at 41°C. in all subsequent experiments. A higher temperature increased the rate of the rhythm, but it was not sustained. At a lower temperature the rhythm decreased and then disappeared.

Rhythmicity and tonus. Practically every preparation studied showed some rhythmicity and tonus which persisted for about 20 minutes; it rarely persisted longer than 60 minutes. Some preparations manifested only a feeble rhythm; yet, they would respond well to drugs. In some preparations the rhythmicity was continuous; in others it was intermittent

or occurred in periods. This latter phenomenon had to be considered in the interpretation of the response of the strip to drugs and ions.

The various types of rhythmicity and the variations in rate and tonus are illustrated in the tracings. Tracings *1* and *2* in figure 1 illustrate the more frequently observed rhythm and tonus changes.

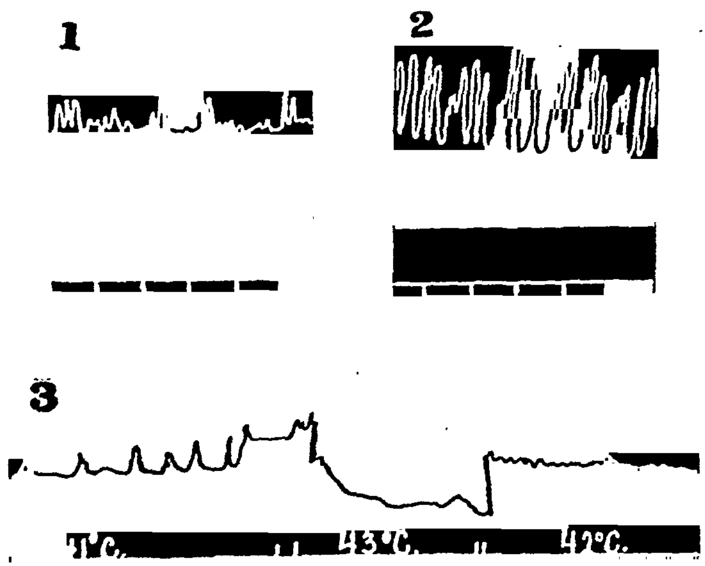

Fig. 1. Tracings *1* and *2* illustrate the variations in rhythmicity and tonus changes observed in the amnion muscle. Tracing *3* illustrates the loss of tone or the relaxation that occurs when the muscle is warmed to 43°C., or illustrates heat paralysis with recovery.

TABLE 1

Showing the rate of rhythmic contractions of the amnion of the chick

DAY	AVERAGE* FIVE-MINUTE INTERVAL	RANGE FIVE-MINUTE INTERVAL	AVERAGE RATE PER MINUTE
10th	34.5	21–60	6.9
11th	34.8	9–53	7.0
12th	16.7	4–36	3.3
13th	20.1	10–31	4.0

* Six specimens for each day.

Incidental observations indicated that the younger specimens were more active. To examine this phenomenon further six strips each were prepared from embryos on the 10th to 13th days of incubation. The results are shown in table 1. The amplitude of the rhythm varied considerably from specimen to specimen, and because of the difficulty of accurately recording

and comparably preparing the strips, the measurements of amplitude were not considered to be worthy of comparison.

When innervated smooth muscle manifesting tone is warmed to 43°C., it elongates (16, 17). When the amnion of the chick was warmed to 43°C., rhythmic contractions disappeared and the muscle elongated. On lowering the temperature to 42°C., the rhythmic contractions returned (tracing *3*, fig. 1). On warming the amnion strip to 46–47°C., the muscle lost its irritability permanently (17). Pierce (2) noted that on cooling the amnion of the chick to 25°C. the tonus of the muscle disappeared and the muscle appeared to be flabby. This observation was readily confirmed; but after the muscle had been cooled to and kept at 22 to 23°C. for five minutes, it would, after a short quiescent period, manifest rhythmic contractions and tonus changes on being placed in the bath at 41°C.

Response to stretch. It could not be demonstrated that a change in tension *per se* influenced rhythmicity and tonus. The mechanical difficulties may have interfered with this experiment. It is possible that by using some more sensitive method of observation a slight response may be detected, although we doubt it. The amnion muscle of the chick is certainly not as sensitive to stretch as the innervated smooth muscle of the intestine and gall bladder (18).

The muscle *in situ* responds to pinching and a wave of contractions sometimes apparently spreads from the point of local contraction.

The response to changes in calcium. Using D-H solution, which contains phosphates, it was found that on increasing the calcium ion from 1.06 m.eq. per liter to 1.54, the tone and rate of contractions were augmented in 4 of 5 specimens. Other experiments were performed with S-R solution, which contains no phosphates. Using the S-R solution, it was found that when the calcium concentration was raised from 2.16 to 2.84 m.eq. per liter, no significant change occurred. Increasing the Ca concentration from 2.16 to 3.02 m.eq. per liter very definitely increased the activity of the muscle. Increasing the Ca concentration from 2.16 to 4.58 m.eq. caused a marked increase, seven experiments being performed. In three other experiments the Ca concentration was raised from 1.62 m.eq. per liter to 2.70; an increase in the activity of the muscle occurred in each instance. Lowering the Ca concentration through the same range decreased the activity of the muscle. These results are similar to those of others working with smooth muscle (15).

The response to changes in potassium. Using S-R solution, the concentration of potassium was raised from the original of 5.1 m.eq. per liter to 5.93 and to 6.375. This change increased the rhythmic contractions, which also occurred when the potassium concentration was raised to 10.05. It was observed that when the initial potassium concentration was 5.1 m.eq. per liter and then the concentration was reduced to 3.18 m.eq. per

liter, the muscle ceased contracting spontaneously and did not recover. Six specimens were used. The changes in concentration were effected as in the case of the Ca studies, by removing an aliquot of the original solution from the bath and then adding the requisite amount of the same type of solution having a higher or lower concentration of potassium so that the concentration of the potassium in the bath would be increased or decreased to the desired extent.

These effects of potassium on the muscle of the amnion are similar to the effects of potassium on the smooth muscle of the uterus (14).

The response to changes in magnesium. The addition of magnesium to the S-R solution, which contains no magnesium, decreased the rate of the rhythmic contractions, especially when the magnesium ion concentration was raised to 2.04 m.eq.; the first definite decrease in rhythm occurred when the magnesium concentration reached 1.04 m.eq. per liter. When the strip was placed initially in D-H solution, which contains 1.0 mM. of magnesium per liter, the magnesium concentration had to be raised to 3.04 m.eq. per liter before the decrease in rhythm was definite. Only three experiments were performed because it is generally agreed that magnesium depresses the activity, but prolongs the rhythmicity of smooth muscle (12, 14).

The response to changes in phosphate. Four specimens were studied in D-H solution in which the phosphate concentration was varied by the addition of either $NaH_2PO_4H_2O$ or Na_2HPO_4. These were added so as to increase the phosphate ion concentration from the initial level up to 3.0 and 4.08 m.eq. per liter. The addition of the acid phosphate increased the activity of the muscle whereas the addition of basic phosphate did not produce a definite change.

The response to drugs. The drugs used were added to the bath which contained 240 cc. either of S-R or D-H solution. The simple addition of 12 mgm. of sodium chloride had no effect on any of the preparations. No drug was added in sufficient quantity to change significantly the pH of the solution. The threshold dose was used to designate that amount of drug which evoked a response in the majority of preparations used. The strip was washed between each application of a drug.

Morphine sulphate. Eleven strips were treated with from 4 to 12 mgm. of morphine sulphate. The threshold dose was 8 mgm. or 1:30,000 concentration. A decisive increase in the rate of the rhythm, in amplitude and a contraction or increase in tone occurred after 12 mgm. or a concentration of 1:20,000. An illustration of a case in which the rate of the rhythm and the level of tone was increased is shown in tracing *1*, figure 2. In no instance was relaxation or inhibition observed.

Baur (6) occasionally observed stimulation with morphine, but reported relaxation to be the predominating response. It is to be noted that a relatively large concentration of morphine is required to produce contraction.

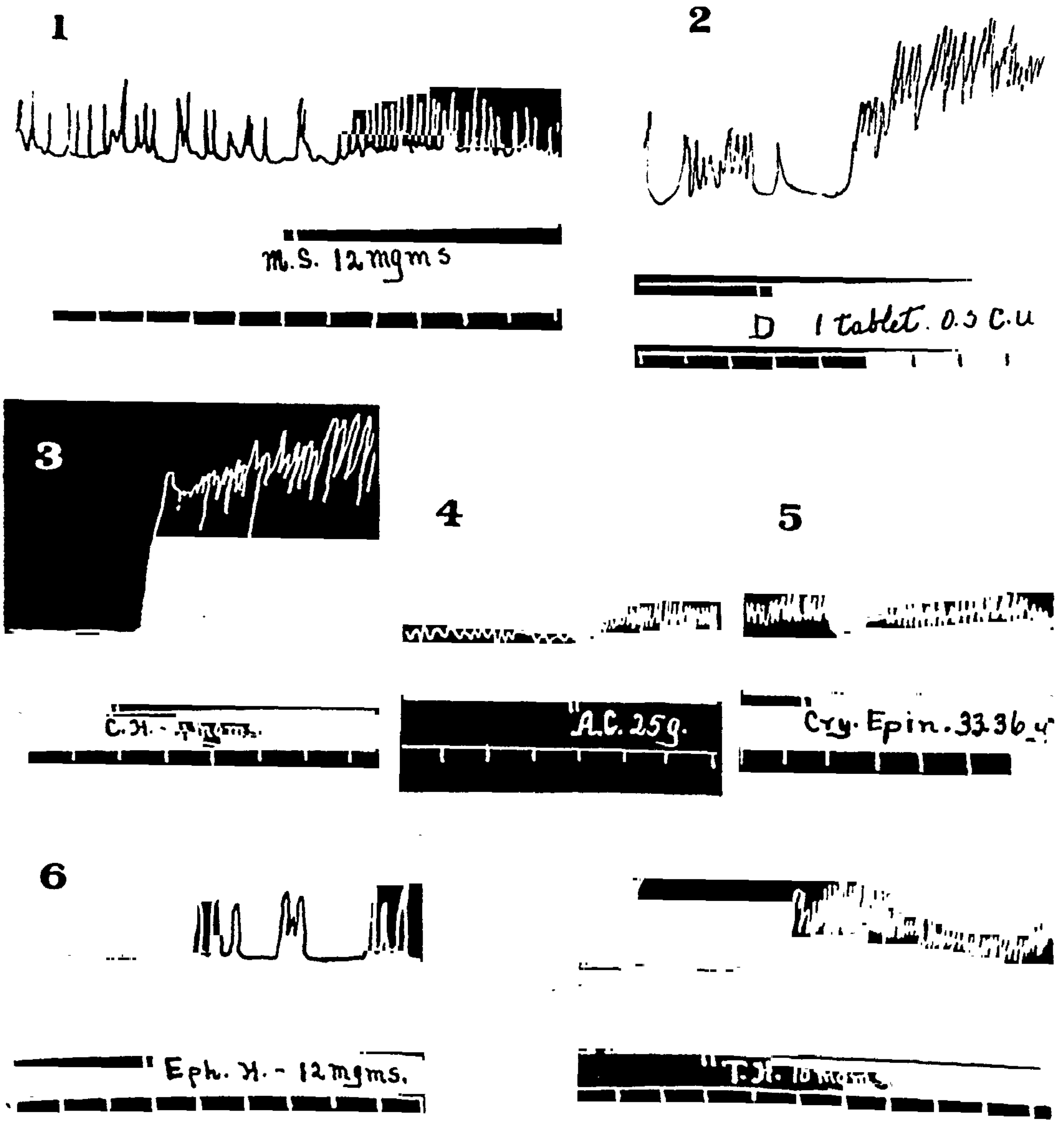

Fig. 2. Tracing *1* illustrates the type of response that occurred to a concentration of 1:20,000 or 1:30,000 of morphine sulphate.

Tracing *2* illustrates the type of response that occurred to 1 or 2 cat units of digalen per liter of solution.

Tracing *3* shows a marked contraction due to cocaine in a concentration of 1:60,000, which is not followed by paralysis if the muscle is promptly washed.

Tracing *4* illustrates increased activity induced by 25 gammas of acetylcholine bromide, or 104 gammas of the bromide or 74.8 gammas of the base per liter of solution.

Tracing *5* shows inhibition caused by 33.36 gammas of crystalline adrenalin, or 139 gammas per liter of solution.

Tracing *6* shows the response of a muscle that had lost its rhythmicity to ephedrine in a concentration of 1:20,000.

Tracing *7* shows the effect of tyramine in a concentration of 1:24,000.

Digalen. This preparation of the water soluble principle of digitalis (19) caused the muscle to contract. The threshold dose was 1.0 cat unit per liter of solution in five experiments. The response of the muscle to approximately 2 cat units per liter of solution is shown in tracing *2*, figure 2.

Cocaine hydrochloride. In 14 experiments it was found that a concentration of 1:480,000 of this drug constituted a dose sufficient to increase the rate and amplitude of the rhythmic contractions in most cases. A concentration of 1:240,000 was required to cause a contraction or an increase in the tonus level. Tracing *3*, figure 2, shows the response of a strip to a concentration of 1:60,000. Larger doses caused paralysis, unless the preparation was immediately washed.

Baur (6) observed only paralysis in response to cocaine, but he performed only two experiments.

Acetylcholine bromide. In 10 experiments the minimum effective dose required to increase the activity of the muscle was 26 gammas (concentration of 1:38,400,000) of the drug per liter of solution. This is equivalent to 18.7 gammas of acetylcholine per liter. The rate of the rhythm and usually the tonus level and amplitude of contractions were increased by that dose. Tracing *4*, figure 2, shows the response to 25 gammas of the bromide per liter of solution.

Acetylcholine bromide and atropine sulphate. The effect of atropine sulphate alone was studied in 10 preparations. The results were variable. A concentration of 1:480,000 of atropine sulphate appeared to stimulate slightly the muscle. The rhythmicity of some strips was inhibited by a concentration of 1:48,000; however, in one instance a concentration of 1:24,000 was required to depress activity and other strips were not depressed even by this concentration.

Using 10 strips it was found that a concentration of 1:480,000 of atropine sulphate abolished the activity induced by 1:120,000 of acetylcholine. After atropine in a concentration of 1:480,000, a concentration of acetylcholine of 1:240,000 was ineffective in causing a contraction. After a concentration of 1:48,000 of atropine, a concentration of 1:48,000 of acetylcholine bromide was ineffective (fig. 3).

It was clear that large doses of atropine were required to inhibit the movements of the strip and even then some were refractory. Much smaller doses antagonized the effect of acetylcholine bromide. No attempt was made to determine the quantitative relations of the atropine-acetylcholine antagonism (32).

Adrenalin. Crystalline adrenalin was employed to determine the threshold dose. In 20 experiments, in which concentrations in the bath of from 1:76,800,000 to 1:120,000 were used, the dose required to temporarily inhibit the muscle was a concentration of 1:9,600,000 or 104 gammas per liter of solution. The response of a strip when 33.36 gammas were

added to the bath is shown in tracing *5*, figure 2. With the threshold dose of 104 gammas per liter the inhibition was definite, but of short duration. Chloretone which is added to adrenalin as marketed, also inhibited the muscle (2); but when diluted to the extent that obtains when commercial adrenalin is usually employed, it had no effect. Hence, the threshold dose of commercial adrenalin on the amnion musculature was found to be the same as that for the crystalline product.

Ephedrine hydrochloride. In 16 experiments, concentrations of this drug were used which varied from 1:240,000 to 1:12,000. The threshold concentration was 1:48,000. It caused an increase in rate, tonus and amplitude. In tracing *6*, figure 2, is shown the response of a muscle to 12 mgm. of the drug, a concentration of 1:20,000. It is to be noted that the muscle was not showing spontaneous contractions at the time the drug was applied.

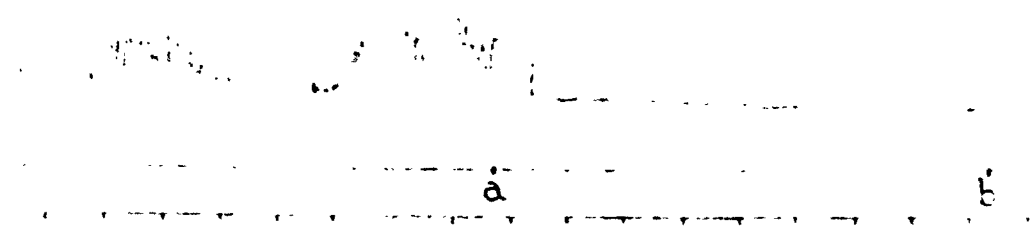

Fig. 3. This tracing shows abolition of rhythmicity and tonus by atropine sulphate in a concentration of 1:48,000, added at *a*. The addition of acetylcholine at *b* failed to cause a significant effect.

Tyramine hydrochloride. In 9 experiments, concentrations of this drug were used which varied from 1:480,000 to 1:24,000. The dose required to increase the rate, amplitude and tonus was 1:80,000. The effect of the drug in a concentration of 1:24,000 is shown in tracing *7*, figure 2.

The effects of cocaine hydrochloride on the action of adrenalin, ephedrine hydrochloride and tyramine hydrochloride. In this group of experiments adrenalin, ephedrine or tyramine was added to determine the responsiveness of the strip. The solution was changed and cocaine hydrochloride was added. Then the original drug was added in the same amount as used prior to the addition of cocaine. Crystalline adrenalin was added in a concentration of 1:240,000 to 1:1,200,000 before and after the addition of cocaine hydrochloride in a concentration of 1:48,000. In every instance adrenalin inhibited the response to cocaine, but the inhibition was less in duration than before the addition of cocaine. No evidence of cocaine potentiation of adrenalin inhibition was obtained. Ephedrine hydrochloride (1:24,000 to 1:48,000) increased the activity before the addition of cocaine, but had no effect after the addition of cocaine. The same was true of tyramine (1:40,000).

In 4 other determinations ephedrine hydrochloride in concentrations of 1:48,000 to 1:24,000 was used both before and after 1:80,000 to 1:48,000 concentrations of cocaine hydrochloride. The ephedrine hydrochloride increased the activity of the muscle before, but had little or no effect after the cocaine was added. Likewise, in 4 determinations with tyramine and tyramine hydrochloride in concentrations of 1:80,000 to 1:40,000 both before and after cocaine hydrochloride in concentration of 1:48,000, the tyramine increased the activity of the muscle before, but had little or no effect after the cocaine.

Thus, after cocaine, adrenalin had its characteristic inhibitory reaction on the amnion muscle. Cocaine did not augment or reverse the effect of adrenalin on the muscle. Cocaine does not reverse the effect of ephedrine and tyramine on amnion muscle. To determine whether cocaine acts synergistically with ephedrine and tyramine smaller concentrations of the drugs will have to be used, since the concentration of the cocaine used may have caused a maximal contraction.

Discussion. The physiological properties of the nerve-free smooth muscle of the amnion, as far as it has been examined, appear to be very similar to those of innervated smooth muscle. One qualitative difference appears to exist, namely, this nerve-free smooth muscle appears not to be stimulated by stretch. In this respect it is analogous to the retractor penis of the dog, which through innervated has no nerve plexus, and does not respond to stretch. To our knowledge no smooth muscle free of a nerve plexus has been shown to be stimulated by stretch (20).

It is frequently stated that such drugs as adrenalin and acetylcholine mimic the action of autonomic nerves by stimulating their nerve endings. Our observations and those of Langley (8) and Baur (6) on the amnion muscle show that this is not necessarily true. Neither is it true for the nerve-free blood vessels of the human placenta (21) nor for the aneural embryonic heart of Fundulus (22) or of the chick (23, 24). Nerve-free smooth muscle may relax (amnion) or may contract (placenta) in response to adrenalin, and may contract (amnion, always) or may relax slightly or contract markedly (placenta) in response to acetylcholine. The response to acetylcholine is antagonized by atropine (amnion, placenta, heart) and augmented by eserine (placenta). The response to adrenalin is abolished by ergotoxine (placenta). Thus these *neuromimetric agents or "chemical transmitters" act directly on non-innervated smooth and cardiac muscle cells.*

The question of the effect of the ingrowth of nerves on the amnion muscle and on the response of the musculature of the blood vessels of the placenta and intestine to drugs can perhaps never be answered. Evidence regarding the question is available for the *heart of Fundulus* (22). Before nerves grow into the heart of this fish, which occurs after it has an otherwise adult

morphology, acetylcholine has a "contracture-like" effect (decrease in diastolic size) which is antagonized by atropine. After the nerves enter the heart, acetylcholine inhibits the heart (in diastole). In this animal the ingrowth of nerves apparently *reverses* and also *sensitizes* the response of the heart to acetylcholine. In the *chick heart*, the ingrowth of nerves apparently does not cause a reversal of the effect of either adrenalin or acetylcholine, but the results with different concentrations of the drugs are so variable as not to permit a conclusion regarding sensitization.

These considerations, along with the greater sensitiveness to these chemicals which apparently occurs after innervation and which would develop a more definite direction to the response and facilitate intercellular conduction (27), would, as Euler (21) has pointed out, harmonize the divergent conceptions of Elliott (26) and Langley (8), regarding the developmental physiology of the autonomic nervous system.

Are excitatory and inhibitory "receptors" for adrenalin and also for acetylcholine inherently present in the same cell? If so, it must be assumed that the adrenalin-inhibitory receptor predominates in the amnion and the adrenalin-excitatory receptor predominates in the placenta. In the case of the heart of Fundulus, the ingrowth of nerves may either sensitize an acetylcholine-inhibitory receptor inherently and latently present, or produce a new receptor, or reverse the one inherently present. Each possibility must be considered and may occur in the adult, in view of the effect of progestin on the functional innervation of the uterus of the cat (27). A developmental pharmacological study of the apparently doubly innervated melanophores of certain fishes would be of interest in this connection, since one nerve contracts and another expands the melanophore (25).

It is clear that atropine, which *per se* has no marked effect on the contractile state of non-innervated cells, antagonizes and prevents the effect of acetylcholine. This shows that atropine opposes the action of acetylcholine by acting on the cell (28, 29) and either the cell must have an atropine receptor, according to Clark (28), or there exists a chemical antagonism between the two drugs, a possibility indicated by Henderson and Roepke (29). That atropine does not paralyze the contractile substance of the non-innervated cell is shown by the fact that cocaine causes a contraction of the atropinized amnion. It therefore may be supposed (21) that atropine in opposing acetylcholine acts on the "fixing power" or *membrane permeability* of the amnion muscle cell.

Cocaine dilates the placental vessels but contracts the amnion muscle. Either the contractile substance or the "receptor substance" differs in the two muscles. The question of cocaine-adrenalin synergism is more involved. One is tempted to conclude that cocaine sensitizes the excitatory but not the inhibitory responses of non-innervated smooth muscle to

adrenalin. But it is known that cocaine sensitizes the innervated bronchial muscle to the inhibitory effect of adrenalin, but not intestinal and urogenital muscle. If the foregoing generalization is made, it is necessary to assume that the ingrowth of nerves into bronchial muscle influences the muscle cell so that the inhibitory effect of adrenalin is potentiated by cocaine. But it is unnecessary to make the additional assumption that in this instance cocaine acts on the nerve ending rather than on the cell, since the ingrowth of nerves may modify the direction or the intensity of the permeability change in the cell membrane induced by cocaine, and we know that cocaine can cause non-innervated smooth muscle to contract or to relax.

SUMMARY

1. The 10th to 14th day amnion of the chick, which is nerve-free, exhibits rhythmicity and tonus changes in a bath of isotonic saline solution. The optimal temperature is about 41°C. At 43°C. it loses rhythmicity and tone but recovers both at 42°C. It survives at 22 to 23°C. for a short period, but loses its vitality at 46 to 47°C. In the saline solutions used rhythmicity rarely persists longer than one hour.

2. The ionic environment influences the motility of this muscle in a manner similar to that observed for innervated smooth muscle.

3. The amnion muscle does not appear to be influenced by stretch. It responds locally to a mechanical and electrical stimulus, and occasionally a wave of contraction appears to be propagated from the point stimulated.

4. The muscle is inhibited by adrenalin (1:9,600,000) and contracts in response to acetylcholine (1:38,000,000) the action of which is antagonized by atropine. Thus these neuromimetic agents act directly on this nerve-free smooth muscle as well as on that of the blood vessels of the placenta (24). The available evidence indicates that the ingrowth of nerves may sensitize and reverse the reaction of smooth muscle to these agents.

5. Atropine in concentrations which antagonize acetylcholine has little or no effect on the amnion muscle; in such doses it appears to stimulate slightly.

6. Adrenalin opposes the contraction of the amnion muscle caused by cocaine, ephedrine and tyramine. Ephedrine and tyramine have little or no effect after cocaine.

7. Morphine sulphate (1:30,000) and digalen (1.0 cat u. per liter) cause the amnion muscle to contract.

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THE RÔLE OF INSULIN IN CARBOHYDRATE METABOLISM

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Two actions are ascribed to insulin in carbohydrate metabolism: that of increasing glucose oxidation and that of increasing glycogen deposition, particularly in muscle. Although such effects are possible, and in some circumstances have been proven to take place, they cannot be used as a basis for an adequate explanation of all the known facts concerning insulin, diabetes, and carbohydrate metabolism. The general literature in this field today stresses particularly the action of insulin in increasing oxidation of glucose, without giving much importance to the increased carbohydrate storing action of this agent. It was our purpose in the work to be presented in this paper to study the relative importance of this second function of insulin in carbohydrate metabolism.

Our first experiments were carried out on depancreatized dogs. The general aim of the work was to determine the relative amounts of insulin needed during the assimilation of carbohydrate and during the post absorptive periods. This latter requirement has been called the "basal insulin requirement" (1). The first dog (table 1) had high carbohydrate feeding days interspersed between fasting days so that the animal practically maintained a steady weight. On the feeding days the attempt was made to give the insulin that was needed for the food. The animal was fed every two hours from 8 a.m. to 8 p.m. and given insulin with each feeding. In the first 3 feeding days, despite administration of 65, 73, 75 units respectively the dog excreted appreciable amounts of sugar in the urine indicating that the insulin was not given in excess. On the 3 first fasting days the dog was given 5, 6 and 5 units insulin respectively and showed urine sugar only on the third day. For the remaining days the urine was kept sugar-free with large doses of insulin on feeding days and small on fasting days. Although the urine showed no sugar on the feeding days the insulin administered could not have been excessive since the dog never had any hypoglycemic attacks. That the dog was kept in a steady state as regards nutrition is indicated by its weight. Furthermore the caloric value of the food given on each feeding day was over double

the daily metabolic requirement that might be expected from the animal's weight. The energy supply of the animal on the fasting days must have come very largely from the sugar and protein fed on the previous day. Since absorption would be practically complete 4 hours after the last feeding we may consider that for 16 hours the animal was absorbing food which was partly utilized then but was mostly put away for use during the succeeding 32 hours. Certainly more of the food would be actually burned during the latter period than during the former, yet by far the bulk of the insulin was needed during the feeding period. The insulin required during the fasting period is no more than the basal need (1).

The point may be raised however that this small amount of insulin needed on the fasting days was necessary for the ultimate combustion of the carbohydrate taken on the feeding days and stored for later use.

TABLE 1

DATE	SUGAR	MEAT	INSULIN	URINE SUGAR	MORNING BODY WEIGHT
	gms.	*gms.*	*units*	*gms.*	*kgm.*
12/ 2/39	500	200	65	4.7	16.7
12/ 3/39	0	0	5	0	
12/ 4/39	700	250	73	14.0	
12/ 5/39	0	0	6	0	17.0
12/ 6/39	0	0	5	13.0	
12/ 7/39	600	550	75	21.0	
12/ 8/39	0	0	6	0	
12/ 9/39	500	100	55	0	17.0
12/10/39	0	0	5	0	
12/11/39	600	200	65	0	16.5
12/12/39	0	0	6	0	16.8
12/13/39	600	300	65	0	16.4
12/14/39	0	0	6	0	

The observations made on the next animal bear on this point. This depancreatized dog (weight 15 K) was given a meal on the first day consisting of 245 grams sucrose and 300 grams meat. (This was the food needed daily to keep the animal in weight balance.) Blood sugar determinations were carried out thereafter at frequent intervals to serve as a guide for the insulin that was required to keep the blood sugar normal. The attempt was made to give just that amount of insulin that was necessary for this.

The results are given in table 2. It is apparent that the animal needed extra insulin from the time of feeding up until about 3 p.m. Thereafter an hourly dose of 0.5 unit insulin sufficed to maintain a steady state. (The duration of action of the larger previous doses is not more than 3 hours (2).) On the next day the animal was fasted and the hourly insulin

requirement needed to maintain a constant normal blood sugar level was found to be 0.5 unit per hour. The same value was obtained on the day following this which was also a fast day. It is reasonable to assume that the source of energy for the period from 3 p.m. to 9 p.m. was the food (predominantly carbohydrate) taken previously. This feeding however was just enough for one day's needs so that by the second day of fasting the animal must have been depending mostly on body fat and a small amount of body protein as a source of energy. Yet the insulin needs are the same for the two periods. That is, the dog required no extra insulin for the extra carbohydrate being metabolized from 3 p.m. to 9 p.m. on the first day. From this it appears unlikely that basal insulin is needed

TABLE 2

TIME (FEBRUARY 8, 1939)	BLOOD SUGAR	INSULIN
		units
8:10 a.m.		4
9:40	Food mixture	
10:30		2
11:15	168	
11:50		2
12:25 p.m.	103	
1:25	114	2
2:25	95	2
3:25	80	1
4:25	75	0.5
5:25	87	0.5
6:25	83	0.5
7:25	87	0.5
8:29	89	0.5
9:25	91	0.5

for oxidation of carbohydrate, but merely to prevent excessive amounts of body protein being broken down to form new glucose (3).

We carried out similar experiments on the depancreatized rat—an animal that does not require basal insulin. The scheme of the experiment was to determine to what extent this preparation could store fed carbohydrate with and without insulin. The high carbohydrate diet given consisted of glucose 68 per cent, Osborne and Mendel salt mixture 4 per cent, commercial casein 18 per cent and "Galen B" vitamin B complex 10 per cent. The vitamin B complex contained 8.6 per cent protein and 59 per cent carbohydrate. The rats had water ad lib at all times. The normal rat is capable of storing fed carbohydrate rapidly since he will maintain steady weight on a regime of a day each of alternate fast and feeding of this diet, as shown in table 3.

The rats were depancreatized by an adaptation of the method of Shapiro and Pincus (4) and the animals were selected for the experiments only if they excreted 8 grams or more sugar per day in the urine on the high carbohydrate diet given above. The animals so selected were subjected successively to 3 regimes, viz: 1, the high carbohydrate diet allowed at all times; 2, alternate days of fast and feeding of high carbohydrate diet; 3, alternate days of fast and feeding plus insulin. The insulin (plain) was administered in 3 doses of 3 units each given at 8 a.m., 2 p.m. and 10 p.m. This amount of insulin was not enough to take care of all the sugar eaten

TABLE 3

Weight response of normal rats to a regime of alternate fasting and feeding with high carbohydrate diet

ANIMAL	NUMBER OF DAYS ON REGIME	WEIGHT AT BEGINNING	WEIGHT AT END
		grams	*grams*
1	10	110	120
2	10	148	159
3	10	104	103
4	10	132	136

TABLE 4

ANIMAL	CONSTANT FEEDING			ALTERNATE FEEDING AND FASTING			ALTERNATE FASTING AND FEEDING PLUS INSULIN		
	Number of days	Weight at first	Weight at end	Number of days	Weight at first	Weight at end	Number of days	Weight at first	Weight at end
A	7	192	198	12	198	170	12	210	204
B	11	152	150	5*	162	122	12	150	150
C	6	170	186	6	172	166	6	186	190
D	8	136	132	8	190	122	8	154	123
E	6	136	140	6	140	101	6	160	140
F	6	153	150	6	164	118	6	194	186
Averages......		156	159		171	133		176	165

* Animal was so weak at end of third fasting period that test had to be terminated.

since the animals always excreted considerable quantities of glucose in the urine. We refrained from giving larger doses from fear of producing hypoglycemia. The results are given in table 4.

The order in which the different animals were subjected to these regimes was varied, in some cases they started on 3 and some on 2. If an animal lost weight on any regime he was given a period of daily feeding plus insulin in order to bring him back to normal before starting the next experimental period. This fact together with the natural growth of the animal during the whole experiment accounts for the differences in the weight of a given animal at the start of the different test periods in some cases.

It is apparent from these results that the tissues of the depancreatized rat can derive adequate nourishment from a high carbohydrate diet and can be kept in a steady state if the animal has access to food at all times. It does not need insulin to utilize sugar. However it has difficulty in storing this food stuff as is indicated by the fact that it cannot maintain its weight if subjected to alternate feeding and fasting periods. It does

TABLE 5

	1	2	3
Constant feeding			
Weight at beginning of period	155	152	138
Weight at end of period	153	154	136
No. of days in period	8	9	9
Alternate fasting and feeding			
Weight at beginning of period	153	127	130
Weight at end of period	132	106	105
No. of days in period	6	4*	4*
Average food eaten, grams per day	29	28	33
Average urine glucose per food day, grams	16.6	11.8	17.7
Average urine nitrogen per food day, grams	0.57	0.675	0.69
Average urine glucose per fast day, grams	0.8	0.6	0.5
Average urine nitrogen per fast day, grams	0.19	0.350	0.20
Alternate fasting and feeding plus insulin			
Weight at beginning of period	132	106	136
Weight at end of period	136	114	132
No. of days in period	6	8	6
Average food eaten, grams per day	26	28	27
Average urine glucose per food day, grams	5.5	4.2	4.7
Average urine nitrogen per food day, grams	0.32	.30	.38
Average urine glucose per fast day, grams	0.9	0.8	1.1
Average urine nitrogen per fast day, grams	0.19	0.19	0.16

* Rats became so weak on third fasting day that regime had to be discontinued.

much better if insulin is given with the feeding which makes it possible for the animal to store carbohydrate to be used in the fasting period.

Three diabetic rats were carried through a series of experiments similar to those already recorded but with additional observations on food eaten, and urinary nitrogen and glucose. The order of the periods for the first two rats was 1, feeding regime; 2, alternate fasting and feeding; 3, alternate fasting and feeding plus insulin. For the third rat 1, feeding regime; 2, alternate fasting and feeding plus insulin; 3, alternate fasting and feeding.

The results are given in table 5. They show the same weight changes as noted in the previous rat series. In the alternate feeding and fasting regime the rats rapidly lose weight if not given insulin with their food. On constant feeding they stay even. They do not eat more when given insulin but rather, somewhat less. The giving of insulin results in a marked reduction of excretion of sugar in the urine, and this "saved" sugar is stored in the body to serve as fuel to carry the rat through the fasting period. The urine nitrogen figures suggest that insulin effected some retention of food protein on the feeding days. This would help to some degree to maintain body weight. However insulin produces no essential difference between the urine nitrogens of the fasting days of the two regimes which indicates that this protein retention did not contribute to energy needs on the fasting day. Although some of the loss of weight on alternate fasting and feeding without insulin was undoubtedly water lost along with tissue loss, it is unlikely that there was loss of weight from dehydration due to glycosuria. The animals had access to water always. Furthermore the high sustained glycosuria of the constant feeding periods resulted in no loss of weight indicating that this did not cause dehydration.

Discussion. Our results indicate that the main disturbance in diabetes is not due to inability to oxidize adequate carbohydrate. The tissues of the diabetic animal are capable of utilizing glucose (5, 6, 7). Our findings support the view that much of the trouble in diabetes due to insulin lack results from the inability of the animal to store carbohydrate. It has been abundantly proven that insulin may increase the storage of carbohydrate as body glycogen. Cori and Cori (8) have studied the effect of insulin on glucose disposal in rats. In this animal under the conditions they used, a large part of the disappearing glucose is deposited as muscle glycogen. Their experiments were of comparatively short duration (4 hrs.) so that the amounts of sugar involved were quite small in comparison with those that we were concerned with in our work. A simple calculation will show that the stored carbohydrate in our rats cannot all be in the form of glycogen. Cori and Cori (8) report that a rat metabolized some 14.5 calories per 100 grams body weight in a day and this would require about 3.5 grams carbohydrate. This amount could in no way be stored as muscle and liver glycogen. Our dog results indicate the same thing. De Nayer (9) working in Boukaert's laboratory used rabbits to study insulin effect. He administered just that amount of glucose that was needed to keep the blood sugars normal. He found that insulin under these conditions disposed of large amounts of glucose, but that there was no increase in muscle glycogen. The glucose must have gone into some other form. Boukaert (10) has suggested that it is

transformed into fat and that one of the important actions of insulin is to increase the conversion of glucose to fat. As pointed out above, the large amounts of carbohydrate that the insulin aids in storing can be accounted for as glycogen only to a small degree. Most of it must be in the form of fat. If insulin has this effect when the body is actually increasing its content of fat it would probably have the same action when the body is in equilibrium as regards fat. This would mean that when the animal is absorbing just enough carbohydrate to supply its metabolic needs, the action of insulin is to convert some of this carbohydrate to fat; at the same time an equivalent amount of fat would be burned.

Such a concept would require a more general transformation of glucose to fat than has been assumed in the present conventional notion of metabolism. According to this teaching it is only when there is a large excess of carbohydrate in the food over that needed for current metabolic needs that it is changed to fat. Such assumptions have resulted from the belief that one has conversion of carbohydrate to fat only when the R.Q. is greater than 1. But the R.Q. of the entire animal gives little indication of the intermediary processes that may be taking place in the different tissues of the animal (11). An R.Q. of less than 1 in an animal on a high carbohydrate diet does not exclude the possibility of the transformation of a part of the carbohydrate of the diet to fat. Such a process could be taking place in some special tissue (i.e., the liver) and if the other tissues of the body were burning an equivalent amount of fat at the same time the resultant R.Q. of the two processes would be the same as that of the direct burning of the carbohydrate. The recent interesting findings of Schoenheimer and his co-workers (12) using deuterium to tag compounds, give support to this concept. They found that in mice that were given heavy water to drink and were on a carbohydrate diet, the fatty acids of the body were replaced after six days by new fatty acids containing deuterium. They also fed mice, in which the depot fat was marked with deuterium, on a bread diet and found the concentration of tagged fat became steadily less, indicating constant new formation of fat. Barrett, Best and Ridcout (13) obtained similar results under these conditions and found the amount of fat in the liver was increased but the concentration of tagged fat here was markedly decreased. We must believe then that there is a much more general conversion of carbohydrate to fat in the normal organism than was formerly supposed. Our findings suggest that this conversion is decreased in diabetes, and that insulin administration increases it. We do not maintain that this is the only action of insulin. It certainly may play an important rôle in the metabolism of other foodstuffs. Thus in some species it is essential for the economy of body protein (3) and for the proper catabolism of fat (14).

SUMMARY

The important action of insulin in carbohydrate metabolism is in bringing about the storage of sugar at the time it is absorbed.

When large amounts of carbohydrate are absorbed by the diabetic organism, a large part of this foodstuff that insulin aids in disposing of must be changed to fat.

Evidence is presented supporting the view that an important action of insulin is to accelerate the transformation of glucose to fat. Without insulin this conversion is either greatly retarded or does not take place at all.

We wish to acknowledge our indebtedness to Dr. Paul O. Greeley for performing the surgery on the animals used in this work.

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THE PRODUCTION OF SHOCK BY THE PROLONGED CONTINUOUS INJECTION OF ADRENALIN IN UNANESTHETIZED DOGS

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Reduced plasma volume is recognized as the central feature of surgical shock. One of the hypotheses (5) advanced to explain the reduction is vasoconstriction which, if of sufficient intensity and duration, leads to anoxia of peripheral tissues, an increase in capillary permeability, and a loss of plasma from the blood stream into the tissues.

The vasoconstrictor concept of shock depends upon the demonstration of a decrease in circulating plasma volume resulting from prolonged vasoconstriction. The present experiments on trained normal dogs were undertaken to determine whether or not a reduction of plasma volume could be produced by prolonged intravenous injection of adrenalin in amounts sufficient to produce a marked reduction in the peripheral circulation.

METHODS. Adult dogs of 12 to 18.8 kgm. were used. They were trained to lie quietly on their sides and were found to remain in this position best while blindfolded. Blood pressure was recorded directly from the left femoral or left brachial artery by means of a cannula, inserted under local anesthesia, connected with a tambour manometer. The rate of blood flow in the right hind paw was measured with a plethysmograph according to the method of Freeman and Zeller (7). Hemoglobin concentrations were measured with a Sahli hemoglobinometer on blood obtained from the right ear. Hematocrit determinations were made on heparinized blood from the right jugular vein at the beginning of the experiment and again at the time when the second plasma volume determination was started.

The plasma volume determinations were made with the dye T-1824 (Gregersen, Gibson and Stead, 10; Gibson and Evans, 8) before the

adrenalin injection was started and again during the injection after one to one and one-half hours. In most of the experiments in which plasma volume was measured, the changes were also calculated from the deviation of the disappearance curve. Exactly 1 cc. of dye was injected into the right saphenous or left brachial vein. Ten to twenty-five minutes were allowed to elapse before taking the first sample. Specimens of blood were taken at regular intervals throughout the experiment in order to follow the changes in the dye concentration. Sufficient blood was taken without stasis from the right jugular vein to yield 2 cc. of serum. In some of the experiments this blood was immediately replaced by citrated blood from a normal dog. The concentration of dye in the serum was measured either spectrophotometrically or by means of a photometric colorimeter (Gibson and Evelyn, 9).

Just before the injection was started, the dogs were given atropin sulphate, 0.2 mgm. per kilogram intravenously, in order to prevent cardiac irregularity. Adrenalin hydrochloride (Parke, Davis and Company) diluted to 1:5000 in physiological saline was used in all but one of the

TABLE 1

DOG	SALINE		BLOOD PRESSURE		BLOOD FLOW		PLASMA VOLUME		
	Time	Total cc.	Initial	During saline	Initial	During saline	Initial	After saline	Per cent change
1	131	91.6	120	130	5.2	9.5		771	
2	78	69.0	115	135	10.0	9.0	879	854	−3
3	97	96.0	120	120	3.0	3.5	845	870	+3

experiments. Powdered adrenalin was made up in physiological saline solution to the same concentration in this exception (see table 2, dog 5). The adrenalin was injected at a constant rate of 0.0034 to 0.0164 mgm. per kilogram per minute. In three additional experiments, an equivalent amount of normal saline solution was injected to determine the effect of the intravenous injection of fluid alone. The injections were made by means of a ureteral catheter inserted under local anesthesia into a large superficial vein of the right foreleg or into the inferior vena cava by way of the left femoral vein. A constant rate of injection was maintained by the use of a Marey flask. The solution was displaced by liquid petrolatum.

Results. When normal saline was injected intravenously the dogs remained quiet, and there were no changes in the mucous membranes or extremities. None of them vomited, and there was no defecation. All recovered uneventfully, and were used in subsequent experiments.

There were no significant changes in blood pressure, blood flow, or in plasma volume, measured before and immediately after the injection. The results are shown in table 1.

Adrenalin. After all operative procedures, including the injection of atropin were completed and before the adrenalin injection was started, the dogs lay quietly with a normal respiratory rate and a rapid, but full, pulse. Shortly after the injection was begun, a moderate hyperpnea was noticed and the dogs became restless. Following this, vomiting frequently occurred.

Usually within ten minutes the mucous membranes of the mouth were seen to become pale and there was gradual progression to cyanosis. At the end of fifty to sixty minutes the clinical signs of shock were marked.

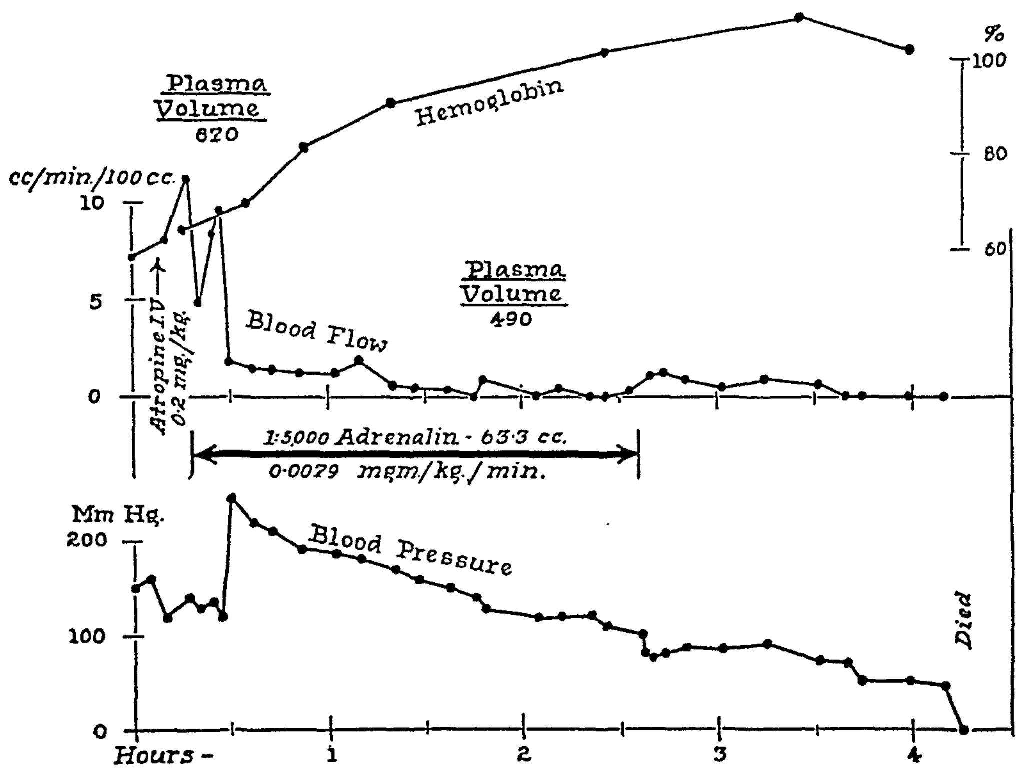

Fig. 1. Effect of prolonged injection of adrenalin (0.0079 mgm./kgm./min.) on blood pressure, blood flow, plasma volume and hemoglobin concentration of unanesthetized dog.

In addition to the pallor and cyanosis, the extremities and ears were cold and had a "doughy" feel. The pulse was thready, and the flow of blood in the ear veins was slow and the blood was dark. The animals were quiet and appeared exhausted but the eye reflexes were active throughout. Involuntary defecation occurred shortly before death.

The rectal temperature rose rapidly, but was prevented from going above 102°F. by wetting the dogs and cooling them with an electric fan.

When the adrenalin injection was started, the blood pressure rose to 250 to 370 mm. Hg. and the rate of blood flow through the paw decreased.

The flow was maintained at a low level during the adrenalin injection, and in most of the experiments it was below one cubic centimeter per minute per 100 cc. paw volume. The blood pressure declined slowly but was maintained throughout the period of injection at a level higher than the normal. The course of a typical experiment is shown in figure 1.

Determinations of plasma volume, made by the "direct" method, showed a fall of from 11 to 44 per cent, averaging 30.6 per cent. Hemoglobin determinations made at the same time indicated a 20 to 70 per cent concentration of the blood. There was no significant change in the plasma protein concentration during the adrenalin injection in the two experiments in which it was measured. The results of all the experiments with injection of adrenalin are summarized in table 2.

TABLE 2

DOG NO.	ADRENALIN	TIME OF INJECTION	BLOOD FLOW THROUGH PAW		PLASMA VOLUME			HEMOGLOBIN		
			Before adrenalin	During adrenalin	Before	After	Change	Before	After	Change
	mgm./kgm./min.	*min.*					*per cent*	*per cent*	*per cent*	*per cent*
1	0.0116	130	10.5	0.5	876	553	−37	85	115	+38
2	0.0129	120	8.8	0.5	1102	758	−31	66	96	+45
3	0.0079	135	8.0	0.7	870	490	−44	64	109	+71
4	0.00465	190	9.6	2.3	1162	820	−29	60	102	+70
5	0.0164	100	9.9	1.9	1062	942	−11	51	61	+19
6	0.0111	126	15.0	3.0	1080	635	−41	98	118	+20
7	0.00766	180	17.2	0.9	627	384	−39			
8	0.0038	210	10.6	2.1	1390	1210	−13			
9	0.0034	166	5.0	1.5				100	166	+66
10	0.0085	132	11.5	0.5				64	87	+36
11	0.0125	66	11.5	0.5				83	136	+64
12	0.0102	141	27.0	0.75				122	168	+38
13	0.0045	143	25.2	1.0				104	152	+46

When the injection was stopped, the blood flow remained at a low level and the blood pressure fell. All but dogs 4 and 13 died or were sacrificed in a few minutes after stopping the adrenalin injection. These two dogs were given transfusions of citrated blood and 50 per cent glucose intravenously. In one hour after the transfusions the blood pressure had returned to the control level and the dogs recovered. The changes in one of these dogs are charted in figure 2.

In five experiments the changes in plasma volume observed with the direct method were followed concurrently by the "indirect" method of Gregersen. As shown in figure 3, according to the values calculated by the "indirect" method, the volume decreased from 1102 to 945 cc. A second

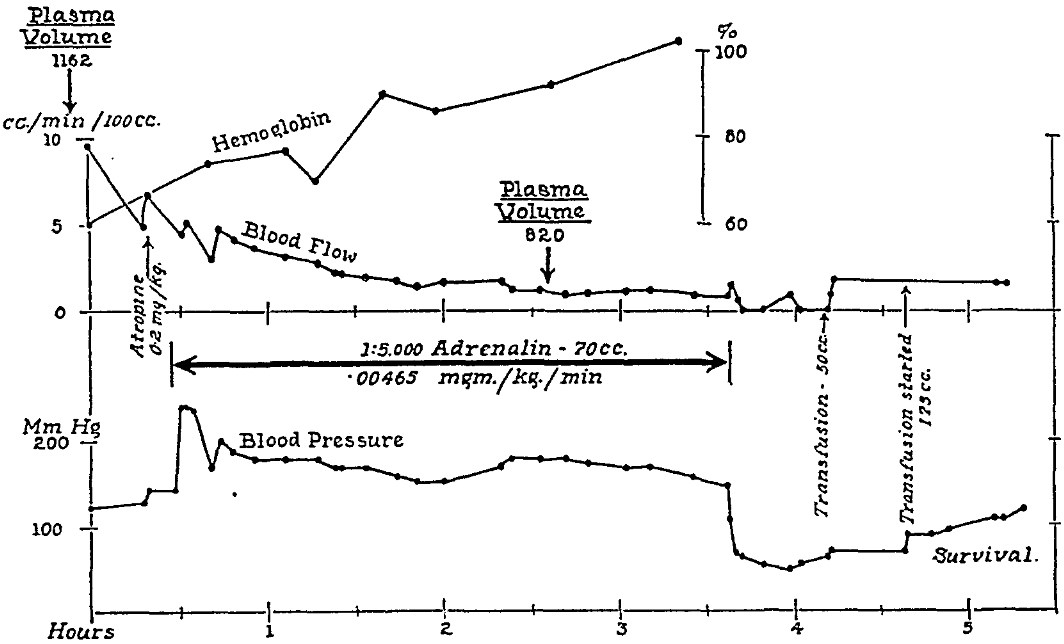

Fig. 2. Recovery of unanesthetized dog from shock produced by prolonged injection of adrenalin after transfusions of blood.

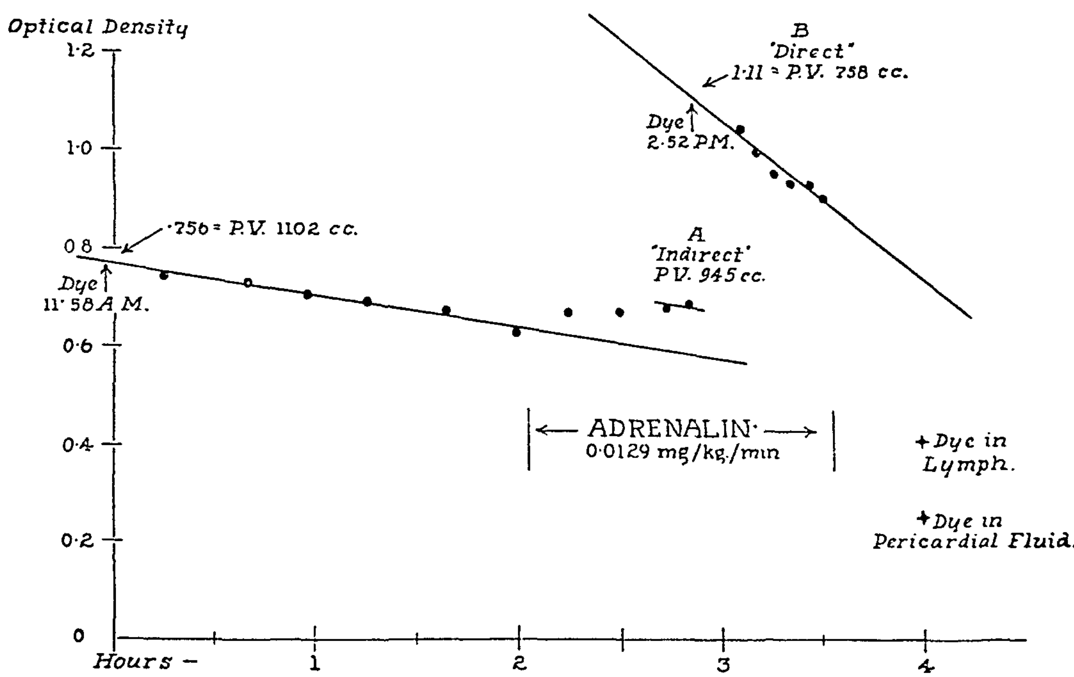

Fig. 3. Comparison of plasma volume changes after prolonged injection of adrenalin: a, by indirect method; b, by direct method after a second injection of dye. Adrenalin (0.0129 mgm./kgm./min.) injected intravenously between arrows. Crosses indicate concentration of dye in lymph from root of mesentery and fluid from pericardial sac.

"direct" determination with a second injection of dye, however, showed the plasma volume to be only 758 cc.

Autopsies were performed on all of the animals. Gross and microscopic changes were consistently observed. The thoracic cavities were empty of fluid, but there was a small amount of clear dye-stained fluid in the abdomen. The lungs were normal both grossly and on section, and showed no evidence of edema, but there were occasional areas of congestion.

The pericardial sac contained small to moderate amounts of dye-stained fluid but there was no evidence of tamponade. The heart was slightly enlarged, chiefly due to dilatation of the right ventricle. There were usually a few petechial subepicardial hemorrhages, and a moderate number of subendocardial hemorrhages over both right and left ventricles. The coronary sulcus was swollen and edematous, but there was no evidence of obstruction to the coronary flow. Section of the heart muscle showed only a small amount of edema.

The gastro-intestinal tract showed the most striking changes on gross examination. The intestines were soggy and the serous surfaces were pale. The lumen of the intestines contained blood-tinged fluid, and on one occasion contained dye-stained fluid. The duodenum consistently had numerous mucosal and submucosal hemorrhages and occasional hemorrhages were present in the stomach and ileum. Dye-stained fluid was obtained from the cisterna chyli on five occasions and in additional instances the mesenteric lymphatics also contained dye-stained fluid.

The liver and kidneys were congested and numerous areas of hemorrhage were present on section. The adrenal medullae were grossly hemorrhagic.

Discussion. The changes observed in the present series of experiments are in agreement with the results of other investigators. Bainbridge and Trevan (1), Erlanger and Gasser (3), Freeman (5), and Lamson and Keith (13) have reported a fall in plasma volume following the injection of adrenalin into anesthetized animals. Gregersen and Pinkston (11) and Hamlin and Gregersen (12), however, have pointed out that the anesthetic alone may cause a fall in plasma volume, and they found no change following injection of adrenalin into unanesthetized animals. They also criticized the method of plasma volume determinations used by Freeman and by Lamson and Keith on the grounds that insufficient time was allowed for mixing of the injected dye in the blood stream. The present series of experiments were carried out on dogs trained to lie quietly without anesthesia. The method of determining plasma volumes described by Gregersen, Gibson and Stead (10) and Gibson and Evans (8) with the dye T-1824 was used. With these methods the injection of adrenalin resulted in a marked fall in plasma volume. Certain observations in these experiments seem to indicate an explanation for the apparent difference in results.

Approximately the same quantities of adrenalin (0.0034 to 0.0164 mgm. per kgm. per min.) were used in these experiments as were injected by Gregersen and Pinkston (0.005 to 0.006 mgm. per kgm. per min.) and by Hamlin and Gregersen (0.005 to 0.035 mgm. per kgm. per min.) in experiments on normal unanesthetized dogs and cats. In our experiments, however, the injection was maintained for one to one and one-half hours before the dye for the second plasma volume determination was introduced. In the experiments reported by Gregersen and Pinkston and by Hamlin and Gregersen, the duration of the injection was less than thirty minutes in two-thirds of the experiments, and exceeded forty-five minutes in only one instance. It seems possible that the discrepancy between our results and those of Gregersen and Pinkston and Hamlin and Gregersen was due to the difference in the duration of injection.

In a personal communication Gregersen pointed out that his experiments on adrenalin injection into unanesthetized animals were designed to demonstrate alterations in normal physiology in contrast to the pathological changes produced in our experiments. None of his animals showed the clinical changes of extreme pallor and cold extremities indicative of intense vasoconstriction, and apparently this effect had not been attained or maintained.

In the present experiments a discrepancy was found between values for the plasma volume determined by the "direct" and "indirect" methods. This discrepancy may possibly be explained on the basis of changes occurring in the circulatory system during the adrenalin injection. The validity of the "indirect" method depends on the assumption that the rate of disappearance of the dye from the blood stream remains constant before and during the injection of adrenalin. However, capillary permeability is probably increased during the vasoconstriction produced by the adrenalin. Landis (14) has shown by direct observation of the capillaries that obstruction to the blood flow and the consequent anoxia of the endothelium results in increased permeability and a loss of both fluid and proteins into the surrounding tissue. For example, he demonstrated (15) that the arterial spasm of Raynaud's disease is followed by an increase in fluid in the tissue spaces. It was observed by Evans and Gibson (4) that where the permeability of the endothelium is altered, the resulting exudates contain high concentrations of the previously-injected dye. Post mortem observations in our experiments have shown dye-stained fluid in the tissue spaces, in the pericardial sac, in the cisterna chyli, and in the lumen of the intestines. Further evidence of rapid loss of dye from the blood stream is seen in the steeper slope of the disappearance curve of the dye following the second injection of dye (fig. 3).

The changes in the heart consistently observed were dye-stained fluid in the pericardium and in the tissue of the coronary sulcus, and subendocardial

hemorrhages. We are unable to explain the mechanism of these changes. However, that they were not significant in causing the death of the dogs was shown by the fact that two dogs recovered following treatment. Post mortem studies in one of these dogs a week later showed evidence of old hemorrhages. Although an increase in venous pressure did occur during the adrenalin injection, as shown by the distended neck veins, it was probably not an important factor in the decrease in plasma volume since the plasma protein concentration was not altered.

A relatively large amount of adrenalin was used, much larger than any amount which could be secreted physiologically. The dosage was chosen to reduce the peripheral blood flow to below 1.5 cc. per minute per 100 cc. paw volume. It was found by Freeman, Shaffer, Schecter and Holling (6) that if the flow was maintained below this level by repeated hemorrhages, shock was produced. The present experiments appear to indicate that if the blood flow is severely reduced from prolonged vasoconstriction due to the injection of adrenalin, shock is produced. The alterations in the physiology leading to the decrease in plasma volume are probably initiated by the anoxia of the peripheral capillaries. The effect of this anoxia is to increase the capillary permeability leading to a loss of fluid and proteins into the surrounding tissues. The blood pressure is maintained by the vasoconstriction but declines slowly due to the loss of circulating fluids. It is the combination of the loss of circulating fluids and the peripheral vasoconstriction which produces the clinical appearance of shock. The central blood flow is maintained as long as the blood pressure remains at a fair level, and the animals remain alert with active reflexes throughout. When the adrenalin is stopped and the blood vessels are permitted to relax, reflex vasoconstriction, in response to the lowered pressure, is insufficient to maintain adequate circulation to the vital centers because of the fall in circulating blood volume.

That the state of the animals during the adrenalin injection was due primarily to the loss of circulating fluids was shown in the two instances in which blood transfusion and intravenous hypertonic glucose solution restored the dogs to normal. Within an hour after this treatment was instituted, the blood pressures had risen to normal levels and the dogs recovered. In another instance, the hemoglobin concentration, which had increased 46 per cent, returned within an hour of treatment to its normal level.

Post mortem studies showed an abnormal amount of fluid outside of the vascular system—in the lymph, in the pericardial sac, in the lumen of the intestine, in the peritoneal cavity, and in the tissue spaces. This finding is considered to be significant. Blalock (2) has suggested that the fluid loss leading to shock is to be attributed to the local congestion, edema, and hemorrhage in the traumatized portion of the body. Frequently, however,

in clinical cases of shock, no such local trauma is discernible, but there is still evidence of a loss of fluid from the circulating blood. The location of this lost fluid has not yet been demonstrated. The findings presented suggest a possible explanation: that the fluid is widespread in the tissue spaces and that some of it is lost through the intestinal mucosa into the lumen of the digestive tract. Improvement following adequate treatment is probably aided by the absorption of this fluid back into the circulating blood.

The changes observed in the experimental animals are much the same as those presented by the human patient in a state of surgical shock. It should be emphasized, however, that there is no evidence that clinical shock in humans is a result of the overproduction of adrenalin. In these experiments, adrenalin was used only as a method of producing prolonged vasoconstriction in the absence of trauma and hemorrhage.

SUMMARY

1. In thirteen experiments on unanesthetized dogs, vasoconstriction produced by the continuous intravenous injection of adrenalin solution (0.0034 to 0.0164 mgm. per kgm. per min.) for a period of one to one and one-half hours resulted in a decrease in plasma volume and a state of shock.

2. Continuous injection of an equivalent amount of normal saline for similar periods caused no change in the plasma volume.

3. The calculation of changes in plasma volume from the deviation of the disappearance slope of the dye T-1824 during the production of shock is not satisfactory because the dye is not retained in the circulating blood.

4. Post-mortem studies suggest that the fluid lost from the circulating blood in shock is distributed through the tissue spaces, and that some of it passes through the intestinal mucosa into the lumen of the digestive tract.

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THE MECHANISM OF THE PRODUCTION OF BRAIN DAMAGE DURING INSULIN SHOCK[1]

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If inhibition of cerebral respiration[2] is the principal, if not the sole cause of the changes which occur during insulin shock, certain inconsistencies must be accounted for. On the basis of experimental work, numerous investigators have suggested other factors to be the cause of brain damage during acute hypoglycemia. These may be summarized as follows: 1. Insulin, per se, is toxic to the brain cell (1, 2). 2. Convulsions cause some changes that produce brain damage (3). 3. Hypoglycemia depresses the metabolism of the brain. This depression causes a decreased oxygen uptake of the brain (4, 5). 4. Insulin shock impairs circulation.

It was shown previously that it is necessary to maintain cats for a definite time in the "medullary phase" of insulin shock (phase IV) to produce brain damage (6). However, an analysis of those results reveals a relationship between the amount of insulin given and the incidence of brain damage.

This report deals with the effects of temperature, sodium pentobarbital, and varying insulin doses on the production of brain damage. Considering the widespread use of insulin shock therapy, and the numerous accidents which occur during its use, a study of this nature seems desirable.

METHODS. Only adult cats weighing 3 kgm. or more, and not previously treated with insulin, were used in these experiments. The method used to produce brain damage has been described in earlier reports (6, 7, 8).

To determine the duration of action of various doses of insulin, blood sugars (Folin-Wu) were taken at intervals. The time for the blood sugars

[1] The author is indebted to Dr. C. H. Thienes for use of the laboratory facilities of the Department of Pharmacology, School of Medicine, University of Southern California, and to the National Youth Administration for the services of Messrs. R. Bernhard, R. Clingman, C. Berry and G. Hogan.

Insulin was kindly furnished by Eli Lilly & Company. Sodium pentobarbital was supplied as Veterinary Nembutal by Abbott & Company.

[2] Inhibition of cerebral respiration refers to the impaired ability of the brain to remove oxygen from the arterial blood during hypoglycemia even though the O_2 saturation may be at normal or near normal levels.

to return to normal was considered the duration of action of the given dose.

In one group of animals 20 u. of insulin per kilogram of body weight were given subcutaneously. When the first myoclonic jerk occurred, 10 mgm. per kgm. of body weight of sodium pentobarbital were given intraperitoneally. The administration of this drug abolished the characteristic progression of symptoms that occur during insulin shock. The only remaining symptoms that could serve as an index of the neurological state of the animal were the respiration and pulse rate. When these became very slow and irregular, the animals were considered to be in the medullary phase. If symptoms of complete circulatory and respiratory collapse became evident, small amounts of glucose were given intraperitoneally, as described in previous reports. In these animals, the hypoglycemias were terminated after they had spent a total of 150 minutes in the medullary phase.

Animals which exhibited the irreversible clinical symptoms of "decortication" or "decerebration," as described by Ziskind and Tyler (7), were considered to have brain damage.

RESULTS. 1. *Sequence of symptoms in the cat during insulin shock.* In cats not receiving sodium pentobarbital the symptoms are similar to those described for humans by Angyal (9) and Frostig (10), the essential difference being the time of occurrence of the various phases. In cats not receiving insulin previously the time before the onset of convulsions increases with the size of the insulin dose given (6). This paradoxical action of insulin does not hold when insulin has been given daily. If the medullary phase occurs, it generally starts from the 6th to the 8th hour after insulin has been given. The number of cats which show medullary symptoms increases progressively with increasing doses (column 2 of table 1). These symptoms rarely occur with doses of 5 u./kgm. or less.

2. *Duration and intensity of action of different doses of insulin.* With 10 u./kgm. (average dose 33 u.), the duration of action ranges from 10 to 14 hours, while with 20 u./kgm. (average dose 70 u.), it is greater than 18 hours (column V of table). These results are in agreement with those found for depancreatized dogs (11). However, the blood sugar level at any instant during hypoglycemia is not an index of the intensity of action at that moment, for the obvious reason that its fall is definitely limited to 0 mgm. per cent. Drury and Greeley (11) measured the intensity of action by means of the ability of various insulin doses to dispose of injected glucose while the blood sugar was maintained at physiological levels. They found that within one hour after giving 10 u. of insulin, a peak glucose disposing ability of 4 to 6 grams per hour was attained by the animal. This gradually tapered off so that at the 3rd hour only 2 grams per hour could be disposed of, and by the 8th hour no glucose

disposing ability was left. With 100 units of insulin a peak of 11 to 13 grams per hour was reached in about 60 minutes. By the 8th hour it fell to 4 to 7 grams per hour, by the 12th hour it could still dispose of from 2 to 4 grams per hour, and, in some instances, the glucose disposing ability still persisted after the 14th hour.

These results indicate that even though the blood sugar level may be the same for different doses of insulin, the intensity of action at the time is dependent upon the amount of insulin initially given. The significance of this in the production of brain damage is discussed below.

TABLE 1

DOSE	HOURS AFTER ADMINISTRATION OF INSULIN THAT MEDULLARY SYMPTOMS OCCUR	NUMBER OF CATS MEDULLARY PHASE OCCURS	INCIDENCE OF BRAIN DAMAGE	DURATION OF ACTION OF INSULIN	BLOOD SUGAR RANGE AT 8TH HOUR	ESTIMATED INTENSITY OF INSULIN ACTION AT 8TH HOUR†
Units per kilogram			*per cent*	*hours*		*grams*
1.0 Av. dose = 3.4		0/8	0	3–5½		0.0
5.0 Av. dose = 16	6¾	1/7	0	6–10	20–43‡	0.+
10.0 Av. dose = 35	7–8	4/7	30*	10–14	18–28	3.0
15.0 Av. dose = 52	6¾–7½	6/6	33*	12–16+	20–30	4.5
20.0 Av. dose = 71	7–8	20/20	62*	18+	18–29	7.0

* From previous report of Tyler and Ziskind.

† Calculated from data of Drury and Greeley, and expressed as the amount of injected glucose that the remaining insulin can dispose of per hour.

‡ Taken on cats still in coma at 8th hour.

3. *The effects of sodium pentobarbital on brain damage.* Of 18 cats given 10 mgm./kgm. of sodium pentobarbital when the first myoclonic jerk or convulsion occurred, 6 died before the hypoglycemias could be terminated. The 12 remaining cats were terminated after they had been in the medullary phase for a total of 150 minutes. This length of time invariably produced brain damage in cats not given sodium pentobarbital, and the resulting preparations usually lived about 48 hours after termination of the hypoglycemia (7). All these 12 cats remained in a protracted coma of from 2½ to 6 days after the hypoglycemias were terminated. Four of these cats died on the 3rd day and 3 on the 4th day without regaining con-

sciousness. One regained consciousness on the 3rd day, showed symptoms of sub-cortical damage, and died on the 5th day. Two cats regained consciousness on the 2nd and 3rd days, and showed symptoms of cortical damage. These cats were sacrificed 21 days later. The 2 remaining cats recovered consciousness on the 3rd and 6th days after termination, and although very sick from a pulmonary infection, did not show any clinical signs of brain damage.

4. *The effects of temperature on the production of brain damage.* When massive doses of insulin are given to a cat, its body temperature usually falls about 6°C. by the time coma sets in. In these "hypothermic" animals brain damage occurs only in the medullary phase (6). If the fall in temperature is prevented, brain damage is produced much more readily. When the body temperature is maintained from 38° to 40°C. the animal gives the appearance of suffering from a severe anoxia. The hypoglycemic symptoms proceed very rapidly, and the animal sinks into the medullary phase suddenly. The character of this phase is altered also. Instead of the usual slow pulse, it may be extremely high. The respiration is essentially Cheyne-Stokes in character. The corneal and pupillary reflexes, as in the hypothermic cat, are absent, and the animal is completely flaccid. Death occurs much sooner in these cats than in the hypothermic cat. If the temperature is raised to above 40°C. during hypoglycemia, death may occur shortly after the animal has had a convulsion. In the hypothermic cat death rarely occurs until the animal has been in the medullary phase for a considerable period. In 3 cats, the body temperature was maintained between 40° and 42°C. by means of a hot pad. The hypoglycemias were terminated one hour after coma set in. All 3 cats showed unmistakable symptoms of cortical damage.

Discussion. From the work of Himwich et al. (5) it is clear that the progression of symptoms during insulin shock is associated with a decreasing ability of the brain to take up oxygen. In other words, as the inhibition of brain respiration increases in severity, lower and lower levels of the brain are affected, depending upon the oxygen need of the various regions. It has been shown that the medulla normally has the lowest metabolic rate (12) and requires the least amount of oxygen per gram weight of tissue (13). It may be assumed, then, that the inhibition of oxygen uptake by the brain must be greatest when the medullary symptoms occur. These symptoms generally appear about the 7th hour (column 1). From the table it can be observed that neither the differences in the duration of action of the various doses of insulin nor the blood sugar levels at the 7th or 8th hours can account for the differences in the incidence of brain damage or the number of animals which show medullar symptoms during insulin shock. In this respect it is found that with 5 u./kgm. the duration of hypoglycemia is long enough to produce medullary symptoms, or even

brain damage, *provided* hypoglycemia, per se, is the only factor involved. Yet, with this dose, only one cat showed medullary symptoms and no irreversible brain damage occurred. On the other hand, with 20 u./kgm. all cats showed medullary symptoms and brain damage was produced in 63 per cent of them. It was pointed out above that the blood sugar level, per se, following massive doses of insulin, is meaningless since it does not give an indication of the intensity of action of insulin at that instant. This "time-activity-factor" is dependent upon the amount of insulin initially given (11). Calculating from the results of Drury and Greeley, we have listed in column VI the estimated amount of insulin action existing 8 hours after injection of different doses. The 8th hour was chosen for these estimations since in the cat it is necessary that the medullary phase be maintained for over an hour to produce brain damage (6). The estimated insulin activity remaining at the 8th hour reveals that with 5 u./kgm., less than 1 gram of glucose disposing ability remains; with 10 u./kgm., it is about 3.0 grams/hour; with 15 u./kgm. it is 4.5 grams/hour, and with 20 u./kgm., it is 7.0 grams/hour. These differences in intensity of action undoubtedly account for the differences in the frequency of medullary symptoms, and also the incidence of brain damage.

Why the inhibition of cerebral respiration is not severest within one hour after insulin is given (for at that time the greatest intensity of action occurs) may probably be attributed to the glycogen reserve in the brain. Although this storage is meager, it is apparently sufficient to take care of the brain's requirement for a few hours (14).

Inasmuch as medullary symptoms indicate an intense depression of the oxygen uptake by the brain (5), and since the frequency of occurrence of these symptoms increases with larger doses of insulin, it may be concluded that the depression of oxygen uptake by the brain ultimately produced depends upon the intensity of insulin action and, thus, on the amount of insulin initially given.

These correlated facts, experimentally established, can account more satisfactorily for the greater incidence of brain damage which occurs with larger doses of insulin than an unknown quality based on the assumption that insulin, per se, is toxic to the brain cell.

Barbiturates are known to depress cerebral metabolism (15). Although it appears from the above results that the degree and incidence of brain damage are less in nembutalized cats, this point is not conclusive. However, despite the occurrence of the prolonged comas, the survival time of the preparations is increased when sodium pentobarbital is given. This drug prevents insulin convulsions but, nevertheless, brain damage will occur. This confirms the findings of others who showed that convulsions were not essential in the production of brain damage (16, 1).

The clinical symptoms of circulatory collapse first occur during the medullary phase. This is as would be expected since the cardiac and respiratory centers in the medulla should be affected by the decreased oxygen uptake at that time. The circulatory collapse, it appears then, is a result of the inhibition of cerebral respiration on those centers, and is an indication that brain damage is taking place.

The mechanism generally accepted to be responsible for the decreased oxygen uptake by the brain during hypoglycemia is based on the observations that the brain can oxidize only glucose or carbohydrate, and when the supply of this foodstuff is curtailed or stopped, the oxygen uptake of the brain is consequently diminished (20, 17, 4, 5). Himwich and his collaborators have further established the close correlation between glucose absorption and the oxygen uptake by the brain. From their work it appears evident that glucose is vitally concerned with cerebral respiration.

If the inhibition of cerebral respiration that occurs during hypoglycemia produces a state very similar to that caused by anoxia, it should be expected that conditions which would lower the oxygen need of the brain should increase the resistance of the brain to damage during insulin shock. In this respect it is found that when the body temperature is allowed to fall during hypoglycemia, the medullary phase has to be maintained for a longer time, in order to produce brain damage, than in those animals whose body temperature is kept at normal levels. Furthermore, it has been noted that precooled animals survive longer when subjected to low partial pressures of oxygen (18) or after receiving lethal doses of insulin (19). Lowering the body temperature lowers the metabolic rate and hence the oxygen need of the tissue. The apparent protective action of nembutal may possibly be explained by the depressed cerebral metabolism that results when this drug is given. Conversely, increased body temperature increases the oxygen need of the brain, and the time required to produce brain damage during insulin shock is shortened.

SUMMARY

Some factors influencing the production of brain damage during insulin shock have been studied. It has been found that:

1. It is necessary to maintain cats in the medullary phase of insulin shock to produce clinical symptoms of brain damage.

2. The medullary phase occurs more often and the incidence of brain damage is greater with increasing doses of insulin. This is attributed to the "time-activity" factor of insulin action whereby the intensity of action of a single dose of insulin at any instant after injection is dependent on the amount of insulin initially given.

3. The length of time that cats must be maintained in the medullary phase of insulin shock to produce brain damage is a function of the oxygen need of the brain.

4. When the body temperature is maintained at normal or above normal levels the medullary phase occurs sooner, the time the animal must be kept in this phase to produce brain damage is shortened and the incidence of brain damage is greater. Low body temperature produces the opposite results.

5. Although prolonged comas result when sodium pentobarbital is given during insulin shock the survival time of the animals is increased.

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THE METABOLISM OF CATS UNDER CHLORALOSE ANESTHESIA, WITH SPECIAL REFERENCE TO OXYGEN CONSUMPTION

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This is to report the variability of, and degree of correlation between oxygen consumption, carbon dioxide output, respiratory quotient, pulmonary ventilation, systolic blood pressure, heart rate, blood sugar and lactic acid concentrations of chloralose anesthetized cats. Such a compilation, though pedestrian and uninspired, may be useful either for: establishment of statistically valid norms and standards as basis for evaluation of experimental results under this commonly used anesthetic; or, perhaps more important, for the insight which is provided, especially by their interrelationships, as to the normality of physiological state and adjustment under anesthesia. In both respects principal interest attaches to oxygen consumption as an index of the total metabolic condition; and it will be analyzed in further detail as to the effect upon it of 1, sex; 2, season; 3, body temperature; 4, body size (weight); and 5, the relation of it and respiratory quotient to values for normal, unanesthetized animals.

Procedure. The data are derived from 50 male and 34 non-pregnant female cats. At the time they were used they had been in the school pens for variable periods of time, but usually not more than a few days. In preparation for experiment they were brought to the laboratory 16 to 18 hours after the last meal and anesthetized with chloralose, 0.1 gram per kilogram, injected under the skin of back or flank. As soon as quiet they were placed on an electrically heated holder and carefully attended to prevent undue lowering or fluctuation of body (rectal) temperature.

Further preparation involved very little trauma, viz., careful insertion of: a tracheal cannula; a blood-sampling cannula in one carotid artery; a blood-pressure cannula in the other carotid; and a cannula in a superficial branch of one femoral vein. Following these simple operative procedures a rest period of at least 15 to 20 minutes was allowed for stabilization before observations were begun.

With 57 of the 84 animals two determinations of respiratory metabolism were made 15 to 20 minutes apart; the second of these duplicate determinations plus the single determination on the remaining 27 animals constitute the 84 observations providing data for part II of table 1. Com-

TABLE 1

Data of cats under chloralose anesthesia

	I				II					
	INTRA-INDIVIDUAL VARIABILITY, COMPARISON OF DUPLICATE DETERMINATIONS, 15–20 MINUTES APART				INTER-INDIVIDUAL VARIABILITY					
	Number	Averages		Diff. per cent of mean	Number	Range	Mean	Standard deviation	Coefficient of variation*	Coefficient of correlation × ———
		1st	2nd							
1. Body weight (kgm.)					84	1.8–4.7	3.0	0.62	20.5	-0.102 ± 0.072 × Pulmonary ventilation -0.099 × O_2 consumption per kilo per min. (partial correlation with temp. constant) -0.029 ± 0.080 × O_2 consumption (entire data) $+0.071 \pm 0.074$ × Blood sugar $+0.114 \pm 0.075$ × Blood lactic acid $+0.123 \pm 0.072$ × Blood pressure $+0.169 \pm 0.070$ × Body temperature
2. Time after injection of chloralose (min.)					84 (73)	60–245 (110–190)	150	32.5	21.7	-0.204 ± 0.068 × Blood pressure -0.087 ± 0.074 × Oxygen consumption -0.085 ± 0.074 × Blood lactic acid $+0.018 \pm 0.074$ × Heart rate $+0.250 \pm 0.072$ × Blood sugar
3. Rectal temperature (°C.)	57	37.7	37.7	0	84 (63)	36.0–38.8 (37.5–38.5)	37.8	0.49	1.29	-0.127 ± 0.072 × Blood pressure $+0.027 \pm 0.076$ × Blood sugar $+0.169 \pm 0.070$ × Body weight $+0.203 \pm 0.071$ × Heart rate $+0.313 \pm 0.070$ × Blood lactic acid $+0.358 \pm 0.064$ × O_2 consumption/kgm. body wt. $+0.382 \pm 0.062$ × Pulmonary ventilation $+0.382 \pm 0.062$ × Respiratory quotient $+0.424 \pm 0.060$ × CO_2 output/kgm. body wt.

4. Blood pressure (mm. Hg)					84	85-235	150	30.8	20.4	−0.264 ± 0.068 × Time after chloralose −0.264 ± 0.069 × Heart rate −0.133 ± 0.071 × Pulmonary ventilation −0.127 ± 0.072 × Rectal temperature −0.016 ± 0.076 × Blood lactic acid +0.086 ± 0.074 × Oxygen consumption +0.123 ± 0.072 × Body weight
5. Heart rate (beats/ minute)					84	95-285	190	34.4	18.0	−0.264 ± 0.069 × Blood pressure +0.018 ± 0.074 × Time after chloralose +0.153 ± 0.070 × Oxygen consumption +0.203 ± 0.071 × Rectal temperature
6. Blood sugar (mgm. per cent)	36	125	129	1.6	78	75-265	145	37.2	25.5	+0.027 ± 0.076 × Rectal temperature +0.100 ± 0.077 × Blood lactic acid +0.112 ± 0.076 × Respiratory quotient +0.250 ± 0.070 × Time after chloralose +0.306 ± 0.070 × Oxygen consumption
7. Blood lactic acid (mgm. per cent)	36	10.8	9.3	7.5	78	4-22	11.2	3.97	36.1	−0.016 ± 0.076 × Blood pressure +0.086 ± 0.076 × Respiratory quotient +0.100 ± 0.077 × Blood sugar +0.232 ± 0.073 × Pulmonary ventilation +0.401 ± 0.067 × Oxygen consumption
8. Pulmonary ventilation (cc./kgm./ min.) (0°-760 mm.)	57	103	102	0.5	84	54-192	107	30.6	28.6	−0.102 ± 0.072 × Body weight −0.133 ± 0.071 × Blood pressure +0.232 ± 0.073 × Blood lactic acid +0.382 ± 0.062 × Rectal temperature +0.457 ± 0.057 × Respiratory quotient +0.729 ± 0.038 × Oxygen consumption +0.781 ± 0.027 × Carbon dioxide output
9. Respiratory quotient	57	0.735	0.741	0.4	84 (72)	0.64-0.86 (0.70-0.86)	0.745	0.048	6.49	+0.086 ± 0.076 × Blood lactic acid +0.112 ± 0.076 × Blood sugar +0.230 ± 0.070 × Oxygen consumption +0.457 ± 0.057 × Pulmonary ventilation +0.532 ± 0.055 × Carbon dioxide output

* Coefficient of Variation $= \dfrac{\text{Standard Deviation}}{\text{Mean}} \times 100.$

TABLE 1– *Concluded*

	I				II						
	INTRA-INDIVIDUAL VARIABILITY. COMPARISON OF DUPLICATE DETERMINATIONS, 15–20 MINUTES APART				INTER-INDIVIDUAL VARIABILITY						
	Number	Averages 1st	Averages 2nd	Diff. per cent of mean	Number	Range	Mean	Standard deviation	Coefficient of variation*	Coefficient of correlation	× ———
10. Carbon dioxide output (cc./kgm./min.)	57	4.65	4.63	0.2	84	2.7–7.7	4.81	1.05	21.9	+0.424 ± 0.060	× Rectal temperature
										+0.532 ± 0.055	× Respiratory quotient
										+0.781 ± 0.027	× Pulmonary ventilation
										+0.948 ± 0.014	× Oxygen consumption
11. Oxygen consumption (cc./kgm./min.)											
(1) Males					(50)	(4.6–9.3)	(6.59)	(1.25)	(19.0)	+0.948 ± 0.014	× Carbon dioxide output
(2) Females					(34)	(3.7–8.4)	(6.14)	(1.17)	(19.1)	+0.729 ± 0.038	× Pulmonary ventilation
(3) Total	57	6.35	6.24	0.9	84	3.7–9.3	6.43	1.23	19.2	+0.401 ± 0.067	× Blood lactic acid
										+0.358 ± 0.064	× Rectal temperature
										+0.306 ± 0.070	× Blood sugar
										+0.230 ± 0.070	× Respiratory quotient
										+0.153 ± 0.072	× Heart rate
										+0.086 ± 0.074	× Blood pressure
										−0.029 ± 0.080	× Body weight, entire series 84 observations
										−0.083 ± 0.084	× Body weight, within temperature range 37.5–38.5 C.
										−0.097	× Partial correlation with weight for the entire group with temperature constant
										−0.087 ± 0.074	× Time after injection of chloralose
12. Composition of expired air (per cent)											
(1) Oxygen	57	14.86	14.88		84	12.05–17.76	14.98	1.10	7.3		
(2) Carbon dioxide	57	4.68	4.72		84	2.68–6.80	4.62	0.76	16.4		

parison of the first and second determinations, when two were made, provide the data on intra-individual variability of part I of the table. Pulmonary ventilation and respiratory metabolism were determined by collection and analysis of expired air.

At the conclusion of the second metabolism determination when two were made, or of the single measurement, when there was only one, record was obtained of carotid blood pressure and heart rate by mercury manometer connected with the carotid pressure cannula. These 84 determinations are thus synchronous with the 84 measurements of respiratory metabolism of part II of the table and are recorded with them there.

With 78 of the 84 animals a blood sample of 2 cc. was taken just preceding the blood pressure determination; estimation of blood sugar and lactic acid concentrations derived therefrom are thus synchronous with the other data of part II of the table and are there recorded. An additional blood sample was also taken at the conclusion of the first measurement of respiratory metabolism with 36 of the 57 animals on which this duplicate determination was made. These 36 pairs of duplicate samples, taken 15 to 20 minutes apart, provide the data on intra-individual variability of blood sugar and lactic acid of part I of the table. Analyses were made on Folin-Wu tungstic acid filtrates; sugar by the method of Hagedorn and Jensen; lactic acid by that of Friedemann, Cotonio and Shaffer.

RESULTS. *Intra-individual stability.* The question that comes first to mind in consideration of metabolism under anesthesia is whether, at the time of the observations, absorption, excretion or destruction of the anesthetic are sufficiently balanced or uniform to permit establishment of a physiological steady state. Most of these determinations were made within the period, 110 to 190 minutes, i.e., essentially during the third hour after subcutaneous injection of the chloralose; and the data of part I of table 1 show that at this time there was practically no change during a 15 to 20 minute interval in any of the variables measured. The data of part II of the table may, therefore, be accepted as characteristic of a stabilized narcotized condition.

Inter-individual variability. All measures of dispersion (range; standard deviation; coefficient of variation) of part II, table 1, are large. Body weight, the time after injection of anesthetic when observation was made, and body temperature, with proper selection and care could have been controlled within narrower limits. It is interesting to see to what extent variation of these may be responsible for the high degree of variability of the others.

Body weight as an index of size might be expected to influence oxygen consumption per unit weight; or, as index of age, blood pressure and perhaps, composition. In so far as degree of correlation (table 1, part II) permits causal inference, it may be eliminated as being responsible in

any significant degree for the wide variability shown by these data. This is not to say that in normal animals or under other conditions it might not be decisive, at least for certain functions; it is merely to point out that under the conditions of these experiments other factors must have played a far more disturbing rôle. The small negative correlation with oxygen consumption per unit weight, which only in sign is indicative of a relationship which might have been expected to be much more pronounced will be referred to later in more detail.

Time after injection of the chloralose when the observations were made might also be suspected as an underlying cause of variability through variation in depth of narcosis. Again, as judged by the coefficients of correlation (table 1, part II) it can have played but a small part. As might have been expected, blood pressure shows a barely measurable tendency to fall, and blood sugar to rise as narcosis deepens or is prolonged. But all correlations are too small to warrant belief that significantly greater uniformity would have been obtained had all observations been made at the end of exactly the same duration of anesthesia.

Body temperature, on the other hand, for uniformity of result, should have been controlled within narrower limits; blood lactic acid level, respiratory metabolism and related volume of pulmonary ventilation are all, according to statistical measure (table 1, part II), affected by it. Though significant, however, all correlations are too small to permit belief that any large part of the observed variability between different animals is due to this cause alone. The fact that these observations were made over the body temperature range observed here permits calculation of temperature coefficients for the functions that are significantly affected. This will be developed for the rate of oxygen consumption below.

From the preceding it may be concluded that variability of these extraneously controllable factors contributed but little to the observed large intrinsic variation of physiological functions in these animals. Next in interest would be to discover, if possible, whether some one disturbing factor in turn affected others to abnormal variability.

Physiological interrelationships under anesthesia are normal, would seem the legitimate conclusion from the observed correlations of which the nearly one-to-one relationship between oxygen consumption and carbon dioxide is pivotal, as the latter might so easily be falsified. The high degree of correlation of carbon dioxide output also with pulmonary ventilation and respiratory quotient would be expected in any case; but is apparently conditioned normally, here, since neither of the last two is significantly related to blood lactic acid level. Nor is the reciprocal relation of pulmonary ventilation to blood pressure large enough to warrant suspicion of suppression of the former from this cause sufficient to interfere with adequate oxygen intake and carbon dioxide output.

With this basis for believing oxygen consumption, however variable, a valid measure of the true metabolic rate of these anesthetized animals, justification is provided for further analysis with respect to the effect upon it of 1, sex; 2, season; 3, body temperature; 4, body size (weight); and finally, 5, its relation, together with that of respiratory quotient, to values for normal, unanesthetized animals.

1. Sex. The influence of this factor persists under anesthesia and therefore contributes something to the range of variability of the group as a whole. For the 50 males the range and average are, respectively, 4.6 to 9.3, and 6.59 cc. per kilo per minute; for the 34 females the corresponding figures are 3.7 to 8.4, and 6.14. The variability as measured by the coefficient of variation is practically the same for each sex, being 19.0 for the males and 19.1 for the females.

2. Season. Some of the variability of the group as a whole is apparently due to the fact that observations were made throughout the year except July and August. Thus the average rates of oxygen consumption, cc. per kilo per minute, with the number of determinations (in parentheses) are: winter, 5.53 (19); spring, 6.95 (38); June, 7.07 (12); autumn, 5.62 (15). The average rectal temperatures for the same seasonal groups are: 37.90; 37.91; 38.05 and 37.36°C.; therefore with the exception of the determinations made in autumn the seasonal difference is independent of this factor; and even for this group the temperature difference is not sufficient, as will appear from what follows, to explain all the decrease in metabolic rate.

This seasonal variation is shown by both sexes; thus, again, rates of oxygen utilization in cubic centimeters per kilo per minute, with the number of determinations (in parentheses) are:

Males: winter, 5.66 (9); spring, 6.93 (27); June, 7.4 (8); autumn, 5.40 (6).

Females: winter, 5.41 (10); spring, 7.00 (27); June, 6.40 (4); autumn, 5.77 (9).

The data are apparently insufficient to decide precisely whether maximum is in spring or early summer, or minimum in autumn or winter, but there would seem to be no doubt of a major cycle throughout the year, and which in these laboratory-confined animals agrees more nearly with that observed in human subjects in bed in the morning without previous immediate exposure to the weather (Gustafson and Benedict, 1928; Lockwood and Griffith, 1938) than with that of those suffering such exposure on the way to the laboratory before measurement (Griffith et al., 1929a). Also, for similar variation in the blood pressure of unanesthetized dogs see Hamilton et al., 1940.

3. Body temperature. The rectal temperatures of these animals varied from 36.6 to 38.8°C.; with 63, or 75 per cent of them within the range of a

single degree, 37.5 to 38.5°C. The average temperature for the entire group is 37.8° which is 0.9°C. below the normal for the cat, 38.7°C.

The coefficient of correlation between rectal temperature and rate of oyxgen consumption is $+0.358 \pm 0.064$; and the regression equation derived from this is:

$$\text{cc. } O_2 \text{ per kilo per minute} = (0.904 \times T°C.) - 27.8$$

According to this, over the two-degree range from 36.6 to 38.6°C., which practically corresponds with the extremes observed in this work, average oxygen intake should vary from 5.3 to 7.1 cc. per kilo per minute; and anesthetized animals held at the normal temperature (for the cat) of 38.7°C., should have an average oxygen consumption of 7.18 cc. per kilo per minute. This will be reverted to below in comparison of these with normal animals.

4. Body weight. Until the test was made it was expected that a good deal of the variability of oxygen consumption per unit of body weight was probably due to use of this convenient but supposedly unphysiological standard of reference. Analysis shows, however, that this has little or nothing to do with the wide variability of result and that there is practically no tendency within this group of anesthetized animals for metabolic rate per unit of weight to be consistently greater in small than in large animals. Thus within this weight range of 1.8 to 4.7 kilos, the coefficient of correlation between weight and oxygen utilization per unit of weight is only -0.029 ± 0.080. Aside from the anticipated negative sign this is numerically too small to have significance.

The relationship is slightly but not significantly improved if allowance is made for body temperature. Thus if all observations at extreme temperatures are eliminated and the correlation is determined only for those (63 in number) within the single-degree range of 37.5 to 38.5°C., it is increased only to -0.083 ± 0.084; i.e., is still no greater than its probable error and therefore of no value. A more complete correction for the possible disturbing influence of temperature is obtained by applying partial correlation to the entire series of data according to the formula:

$$r_{12.3} = \frac{r_{12} - r_{13} \cdot r_{23}}{1 - r_{13}^2 \cdot 1 - r_{23}^2}$$

where r_{12} = correlation between weight and oxygen per unit weight
r_{13} = correlation between temperature and oxygen per unit weight
r_{23} = correlation between temperature and weight.

This gives a value of -0.097, which again is no indication of well-defined relationship.

The entire data were then recalculated on the basis of weight to the two-

thirds power; which, as is well known, is more nearly proportional to body surface; with, if anything, greater dispersion; the results being:

Range = 4.5 to 14.0 cc. per kgm.$^{\frac{2}{3}}$ per minute.
Standard deviation = 1.86
Coefficient of variation = 20.1

It seems impossible, therefore, to escape the unexpected conclusion that these anesthetized animals do not obey the surface-area rule; and that although their metabolic rate is not very constant per unit of weight it is no more constant when reduced to a unit proportional to body surface.

5. Comparison with normal, unanesthetized cats. Oxygen consumption. Data for this use are not too abundant; some may be found in the publications of Aub, Bright and Uridil (1922) and of Hunt and Bright (1926); our own are as follows and consist of 32 observations on 7 normal male cats. With two of these the respiratory metabolism was determined with

TABLE 2

Data of normal unanesthetized cats

FUNCTION	NUMBER OF DETERMINATIONS	RANGE	MEAN	STANDARD DEVIATION	COEFFICIENT OF VARIATION
Body weight (kgm.).....	7	2.5- 3.87	3.13		
Oxygen consumption (cc./kgm./min.).......	32	4.3-11.8	6.74	2.07	30.7
Carbon dioxide output (cc./kgm./min.).......	32	3.7- 9.6	5.81	1.40	24.1
Respiratory quotient....	32	0.62-0.88	0.746	0.067	8.98
	(8)	(0.62-0.70)			
Alcohol checks..........	14	0.644-0.665	0.657		

a Benedict type of closed-circuit apparatus; with the other five Haldane's open-circuit method was used. The results are shown in table 2.

Comparing the rates of oxygen utilization of our normal (6.74 cc. per kilo per minute) and anesthetized (6.43 cc. per kilo per minute) cats there is an apparent decrease due to the anesthetic of 0.31 cc. per kilo per minute, or 4.6 per cent. It is necessary, however, to make allowance for the difference of body temperature. As mentioned in a previous section, calculating on the basis of the temperature effect observed in these anesthetized animals, at normal (cat) temperature of 38.7°C. their average rate of oxygen utilization could be expected to be 7.18 cc. per kilo per minute; this is 0.44 cc. per kilo per minute, or 6.5 per cent above the observed average for the normal, unanesthetized animals of 6.74 cc. per kilo per minute.

We thus arrive independently at the conclusion previously reached by Aub, Bright and Forman, and by Hunt and Bright: viz., metabolic rate is increased under anesthesia; the increase of 6.5 per cent under chloralose

is a less abnormality than the elevation of "over 10 per cent" reported by Hunt and Bright for amytal, or the 15 per cent increase found by Aub and his co-workers for urethane. By the latter this was attributed to stimulated activity of the adrenals; Hunt and Bright considered this less probable under amytal; only further work can determine whether the same cause is responsible in the three different instances and exactly what it is.

It may be pointed out, in conclusion, that the coefficients of variability for oxygen consumption (30.7), carbon dioxide output (24.1) and respiratory quotient (8.98) are of the same order of magnitude, but even greater, in these normal animals than they are in the anesthetized condition. This is not surprising in view of the difficulty of obtaining strictly basal states in an active, normal cat; it might have been expected, however, that a greater uniformity would have obtained in the complete quiesence of anesthesia. At least, however, the variability under chloralose cannot be attributed in any large measure to the action of the anesthetic; but rather to its inability to suppress in any considerable degree normal individualities of metabolism.

The respiratory quotient. Aside from its quantitative effect it might appear from the low quotients observed in these anesthetized animals that chloralose produced qualitative changes in metabolism. It will be recalled that the range of the 84 determinations was from 0.64 to 0.86, and the average, 0.745; with 12, or 14 per cent, of the values below 0.70. Referring to the data for normal cats (table 2), however, it will be seen that almost precisely the same condition obtained with them: the 32 determinations had almost exactly the same range (0.62 to 0.88) and average (0.746); and 8, or 25 per cent, were below 0.70. The range (0.644 to 0.665) and average (0.657) of the alcohol checks run concurrently with these determinations and inspire confidence that they are probably not too much in error. It would appear, therefore, that chloralose has no specific effect on qualitative metabolism; nor does the cat seem more prone to development of low quotients in the post-absorptive state than the rabbit (Lee, 1939).

SUMMARY

Data are given describing the range, mean, standard deviation, coefficient of variation, and interrelationships, as established by the coefficient of correlation, for: blood pressure and heart rate, blood sugar and lactic acid, pulmonary minute volume, carbon dioxide output, oxygen consumption and respiratory quotient of 84 cats under chloralose anesthesia; and, in addition, for comparison, 32 determinations of: oxygen consumption, carbon dioxide output and respiratory quotient of 7 normal unanesthetized cats.

During the second to fourth hour of anesthesia all of these physiological factors are practically unchanging over short experimental periods of 15 to 20 minutes' duration.

Inter-individual variability of all functions (range and coefficient of variation) is large; the variations appear, however, to be normally interrelated (coefficients of correlation), indicating that chloralose produces no grossly abnormal physiological disturbance; in particular the respiratory quotient appears unaffected.

Oxygen consumption of the anesthetized animals is scrutinized in more detail and is shown: 1, not to be affected by duration of anesthesia within the time limits employed here; 2, nor to owe its variability per unit of body weight to inverse relationship with this factor; 3, to vary according to sex, season and body temperature; and 4, to be increased approximately 7 per cent by the anesthetic.

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THE ELECTRICAL EXCITABILITY OF THE SUPERIOR CERVICAL GANGLION OF THE CAT

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While much precise information has accumulated concerning the excitability of nerve axons, little is known about this aspect of the other parts of the neuron, namely, the cell body and the dendrites. In the oculomotor neurons Lorente de Nó (1935) demonstrated that the cell body (including the dendrites) is electrically excitable. The present work was intended to reveal whether or not the same is true of the postganglionic sympathetic neurons whose cell bodies lie in the superior cervical ganglion.

The method employed was the comparison of excitability curves of the pre- and postganglionic nerves with similar curves taken with the stimulating electrodes in a favorable position for the stimulation of the ganglion cells. The curves studied were either voltage-capacity or voltage-response curves. For multifibered nerves these curves are commonly found to be continuous.

Lucas (1907a, b) was the first to point out that a discontinuity in the strength-duration curve might indicate that the organ contained two or more groups of excitable elements with different characteristics of excitation. By comparing the curves for nerve and for nerve-free muscle with that for innervated muscle, he was able to identify in the complex curves of the latter the components contributed by nerve and by muscle. Hill (1936) and Maltesos and Schneider (1938) found breaks in the strength-duration curves of nerve trunks; and these cases they attributed to a grouping of nerve fibers about two or more levels of excitability much as Lucas had found for nerve and muscle.

Voltage-response curves describe the distribution of thresholds of electrical excitability of the elements in the field of stimulation in terms of a response which is cumulative as the voltage increases above the lowest threshold. Here breaks in the smoothness of the curve would denote grouping of relatively more elements at certain thresholds than are present at others.

The superior cervical ganglion resembles the innervated muscle in Lucas' experiment. It includes both preganglionic axons, corresponding to Lucas' "nerve," and postganglionic neurons, corresponding to Lucas'

"muscle." The excitability curves of the preganglionic axons can be tested caudad along the cervical sympathetic trunk, and those of the postganglionic axons can be tested cephalad to the ganglion. The excitability curve of the ganglion, like that of Lucas' innervated muscle, might be expected to contain a break, marking the crossing of the curves of these two components of the ganglion.

The ganglion may, however, be simplified by section and degeneration of the preganglionic axons. It now contains only the various parts of the postganglionic neurons (dendrites, cell bodies and beginnings of axons). Any difference between the excitability curves of the ganglion and those of the postganglionic nerve can be attributed to the special features of the ganglion. In particular, given smooth curves for the postganglionic nerves, a break in the excitability curves of the ganglion suggests that the ganglion contains two groups of electrically excitable elements, one of them the postganglionic axons and the other, therefore, one of the other parts of the postganglionic neuron, either the ganglion cells or their dendrites.

METHOD. Cats were used under dial anesthesia (Ciba, 0.8 cc. per kgm.). "Denervated ganglia" were those whose preganglionic nerve trunk had been sectioned aseptically 1 to 4 weeks previously. The complete degeneration of the nerve was checked in the acute experiments by failure of response of the iris and nictitating membrane on tetanic stimulation of the remains of the nerve.

Care was taken to preserve the integrity of the ganglion during operative procedures. Nerves IX, X, XI and XII were sectioned centrally and peripherally to the region of the ganglion to avoid the effects of spread of stimulating current. Pairs of chlorided silver electrodes, each less than 0.5 mm. in diameter, were placed at the cephalic and caudal tips of the spindle-shaped ganglion. The caudal pair served for preganglionic, the cephalic pair for postganglionic stimulation, and one of each for stimulation of the ganglion itself. The electrodes of the pre- and postganglionic pairs were separated by 2 to 5 mm., while the distance between the electrodes adjacent to the ganglion was 6 to 15 mm.

Condenser discharges of known capacity and voltage were used for stimulations, the cathode being cephalad. In some animals a constant frequency (1 to 5 per second in different experiments) was applied for a definite period or until the response had reached a plateau. With this mode of stimulation isotonic contractions of the nictitating membrane were recorded on a smoked drum by a light lever giving a magnification of about 20 times. In other animals single shocks were applied and the excursions of a beam of light were observed as reflected to a millimeter ruler from the mirror of a frictionless torsion spring myograph registering tension of the nictitating membrane. The greatest excursion of the free

border of the nictitating membrane in the latter case was less than 2 mm., and the magnification by the beam mechanism was about 60 times. In any experiment the interval between stimulations was fairly constant.

Responses of the nictitating membrane were recorded for different voltages at the same capacity (voltage-response curves), or voltage-capacity curves were built for a response of given height, usually 30 to 60 per cent of maximal (Rosenblueth and Rioch, 1933). Double logarithmic plotting was adopted for the latter. For voltage-response curves, the following procedure was adopted in order to check their accuracy: first, a few random voltages were applied in order to estimate the range of the curve; next, a series of 10 or more points was taken with increasing voltages from a very small to an almost maximal response; then, a second more detailed series was taken from higher to lower voltages. Sometimes three or four such series were taken in alternating direction, up and down the voltage scale.

RESULTS. A. *Pre- and postganglionic nerves.* Some observers (Rosenblueth and Rioch, 1933; Knoeffel and Davis, 1933) have reported smooth voltage-response and voltage-capacity curves for the pre- and postganglionic nerves. Maltesos and Schneider (1938), however, report that in some of their experiments there were breaks in the voltage-capacity curves of the cat's cervical sympathetic nerve. The observations of the latter workers were not confirmed in the present work, since all curves for the nerve-trunks were smooth (fig. 1), even in animals whose ganglion curves (sections B and C) showed breaks.

B. *Normal ganglion.* In 11 normal animals reliable ganglion curves were built. While 2 of these showed smooth voltage-response curves similar to those of the pre- and postganglionic nerves (section A), in the other 9 the voltage-response curves were complex, as shown by breaks. Two of these curves had one break, and each of the remaining 7 showed two breaks (fig. 2). Two voltage-capacity curves were built; of these 1 showed one break, the other, two breaks (fig. 3).

C. *Denervated ganglion.* In 8 animals reliable curves were built for denervated ganglia. Of 7 voltage-response curves, 3 had one break and 4 had two breaks (fig. 4). Of 2 voltage-capacity curves, 1 had one break and 1 had two breaks.

D. *Ganglion destroyed.* After the building of ganglion curves showing breaks (sections B and C), the ganglion between the electrodes was crushed (in 1 animal) or extirpated (in another) and curves were built, using the same electrodes formerly employed for ganglion curves. The voltage-response curves were now smooth.

E. *Variability of response.* Spontaneous variations of the length or tension of the nictitating membrane were frequently encountered in animals with denervated ganglia. The eyeball was removed and the striated

muscles of the orbit were cut to decrease these changes. During the construction of curves reported above spontaneous activity was minimal.

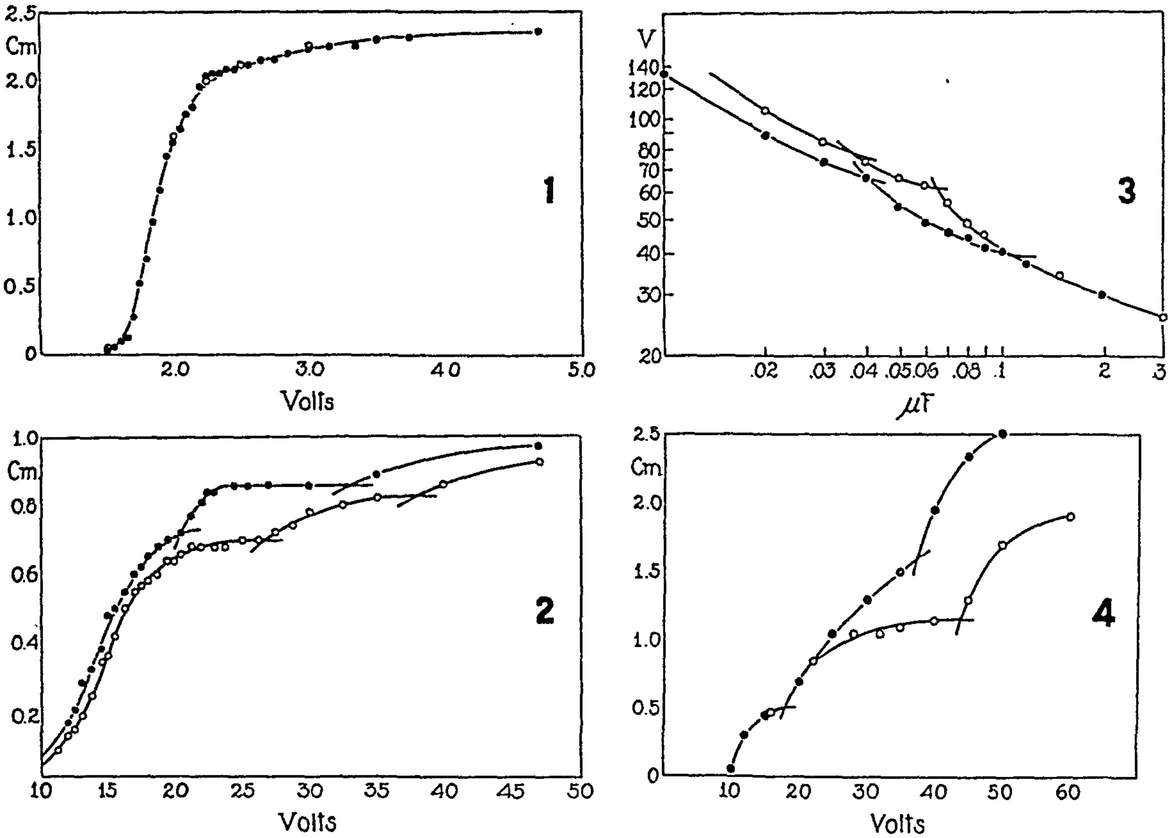

Fig. 1. Cat under dial anesthesia. Voltage-response curve of preganglionic nerve. Each point represents a response of the nictitating membrane to 10 condenser discharges (0.1 μ F) at 4 per sec. Ordinates: volts. Abscissae: response in centimeters.

Fig. 2. Voltage-response curves of superior cervical ganglion. Ten condenser discharges (0.1 μ F) at 4 per sec. Dots: earlier curve, ascending the voltage scale. Circles: later curve, ascending the voltage scale.

Fig. 3. Voltage-capacity curves of the superior cervical ganglion for 2-cm. responses of the nictitating membrane to condenser discharges (5 per sec. for 30 sec.). Dots: earlier points. Circles: later points. Ordinates: volts. Abscissae: microfarads. Double logarithmic plotting.

Fig. 4. Voltage-response curves of denervated superior cervical ganglion. Single condenser discharges (0.02 μ F). Dots: descending the voltage scale. Circles: ascending the voltage scale.

It was usually found in building ganglion curves that after one curve had been completed a repetition of the procedure yielded a curve similar in shape, but shifted along the voltage scale. Figure 4 illustrates this change; first a descending and then an ascending series of voltages were

applied. This shift represented in most cases a decrease of excitability but in some, an increase.

DISCUSSION. The essential fact reported is that excitability curves taken with the electrodes favorably situated for the stimulation of ganglion cells have a more complex configuration than do the curves of the pre- and postganglionic nerves, in that the ganglion curves show breaks while the latter are smooth. This is true even when the ganglion is simplified by removal of one set of its elements, the preganglionic axons. These facts indicate that the neurons connecting with the nictitating membrane have different characteristics of excitation when the ganglion is stimulated from those when the postganglionic axons are stimulated.

Maltesos and Schneider (1938) argued from their finding of breaks in the voltage-capacity curves of preganglionic nerve trunks that the axons serving the nictitating membrane are grouped about two or more levels of excitability. The same explanation might be applied to the breaks in ganglion curves reported above, namely, a grouping about several levels of excitability of the ganglion cells serving the nictitating membrane. In the present work, however, the smooth curves of the postganglionic nerve reveal that the axons are not so grouped, even in animals showing breaks in ganglion curves. This explanation can therefore be applied to the breaks in ganglion curves only if the ganglionic parts of the neurons have different characteristics of excitation from those of the axons. As was concluded above, this condition itself accounts for the breaks when the postganglionic curves are smooth.

The most likely characteristics of the ganglion to account for the singularities of its excitability curve are the anatomical features of the neurons in the ganglion which are absent in the postganglionic trunk, namely, the cell body and the dendrites. The data suggest the presence in the denervated ganglion of three groups of excitable structures, each represented by a segment of the doubly-broken curve (fig. 4). One of these components undoubtedly represents the postganglionic axons. This leaves two components of the curve to represent two other groups of excitable structures in the strictly ganglionic portion of the neuron, i.e., that part which includes the cell body and the dendrites. Assignment of a segment each to these two structures would be mere speculation. The present data do, however, lead us to the conclusions that some part of this strictly ganglionic portion is electrically excitable and that the characteristics of excitation of this part differ from those of the postganglionic axon.

SUMMARY

1. Voltage-capacity and voltage-response curves were built for the superior cervical ganglion of the cat and for its pre- and postganglionic nerves. Responses of the nictitating membrane were used as an indicator of the number of elements excited.

2. In confirmation of the work of Rosenblueth and Rioch (1933) and of Knoeffel and Davis (1933), but at variance with the results of Maltesos and Schneider (1938), these curves for pre- and postganglionic nerve trunks are found to be smooth (fig. 1, p. 575).

3. Curves built with the electrodes at the cephalic and caudal tips of the ganglion present one or two breaks. This is true both of the normal ganglion (figs. 2 and 3) and of the ganglion which has been denervated by previous section and degeneration of its preganglionic fibers (fig. 4; p. 575).

4. It is concluded that the ganglionic part of the postganglionic neuron, namely, the part which includes cell body and dendrites, is electrically excitable and has different characteristics of excitation from those of the postganglionic axon (p. 576).

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ELIMINATION OF SODIUM IN PANCREATIC JUICE AS MEASURED BY RADIOACTIVE SODIUM[1]

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Recent developments in the use of radioactive isotopes have clearly established their value as tracers in the study of movements of elements and compounds in the animal body. The application of tagged elements to the study of the activities of the glands of the gastrointestinal tract would appear to offer certain advantages, since the high degree of radioactivity which may be induced in a small amount of material makes possible the introduction of such minute amounts of labeled salts that they need not alter the normal physiological state of the animal. In addition, the sensitivity of the method available for the detection of radioactivity is such that the labeled salt may be accurately followed, even when only a small fraction of it makes its appearance. The present investigation deals with the secretion of labeled sodium by the pancreas.

EXPERIMENTAL. *Preparation and care of animals with pancreatic fistulae.* Large dogs were used throughout this study. The upper duct of the pancreas was ligated and the lower duct cannulated by a modification of the procedure of Elman and McCaughan (1). A soft rubber tube 7 inches long was used intra-abdominally, while a heavier walled tube 6 to 7 inches long was brought out through a left-upper rectus stab wound. A small tin foil-coated cellophane disc was placed over the glass connector between the two rubber tubes and acted as a stop to prevent extrusion of the rubber tubes through the stab wound. The soft rubber tubing, the cellophane disc, and the first portion of the heavy rubber tubing were wrapped in the distal 1 inch of the omental margin, beginning at a point 3 inches from the pylorus and extending to the neighborhood of the spleen. This produced a gentle semicircular curve of the tubing from the cannula to the stab wound, a procedure that tends to reduce the danger of kinking. The omentum also served to seal the cannula-duct area and to plug the peritubular region at the stab wound, so that no infection could be introduced

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into the general abdominal cavity. The capacity of the tubular system was approximately 2 cc. For the purpose of collecting the pancreatic juice at the times when the animal was not under observation, a sterile rubber bag was attached to the tubing.

The fistulae dogs were maintained in good condition by replacement therapy and an adequate diet. Each animal received daily 1 to 2 liters of Ringer's solution intravenously to insure an adequate supply of salts and liquid. The dogs were fed a basic diet of lean meat and beef lungs. Vitamin supplements were added: A and D in cod liver oil; the B complex in a concentrate obtained from rice bran. Each animal also received 100 grams or more of raw pancreas daily. It has been repeatedly observed here that the daily feeding of the raw glandular tissue provides for a satisfactory nutritional state in dogs with pancreatic fistulae.

Collection of pancreatic juice. Dogs that possessed a satisfactory secretory mechanism on the day of the experiment were accepted as adequate preparations. Stimulation to secretion was produced by the ingestion of 200 grams or more of lean meat 0.5 to 2 hours before the commencement of the collections and 100 grams of meat at intervals thereafter. The dog was laid on its left side with the rubber tubing hanging over the edge of the table. An initial control collection was made 10 to 20 minutes before the introduction of the radioactive sodium into the vein. Then 2 cc. were collected and discarded as representing the residual content of the tubal system. The juice was collected in graduated centrifuge tubes, measured and sampled. Collections were made at approximately 3, 7, 10, 15, 20, 25, 30, 45 and 60 minutes, and at 0.5-hour intervals thereafter for 8 to 9 hours. The animal was then returned to its cage for the night with the rubber bag attached. The following morning the juice that had accumulated during the night was measured and sampled. A final sample was then obtained by collecting the secretion produced during the next 10 to 15 minutes.

Collection and preparation of blood serum. Blood samples were removed from the jugular or leg veins at intervals during the course of the experiment. In no instance was blood taken from the same vessel in which the labeled sodium was injected. Three to 6 cc. of blood were removed at a time, allowed to clot firmly, and then centrifuged at 3000 RPM for 15 minutes. Serum was separated as soon as possible after the removal of blood from the animal.

Preparation of labeled sodium. A layer of metallic sodium was spread over the surface of a copper plate that had perviously been cleaned with concentrated nitric acid. This target was then subjected to a deuteron bombardment in the Berkeley cyclotron. A combination of scraping and washing with 50 per cent alcohol was employed to remove the bombarded sodium from the target. The first step in the purification was to acidify

the solution with HCl and filter out any material which did not dissolve. The solution was evaporated to dryness on a steam bath and then heated with an open flame to insure removal of all traces of alcohol and HCl. To eliminate copper and other heavy metals which might be present, the residue was dissolved in distilled water and the solution saturated with hydrogen sulfide. The sulfide precipitate was filtered out and the excess hydrogen sulfide washed from the solution by bubbling a stream of air through the filtrate. After determining the weight of NaCl present, the salt was dissolved and made up to a 1 per cent solution. A small portion of this solution was evaporated on blotter paper measuring 3.5 x 6 cm. The blotter was then wrapped and sealed in cellophane and an assay of its radioactivity made by a Geiger-Müller counter, which had previously been standardized against a sample of uranium so that the number of emanations detected by the counter per unit of time could be converted into millicuries.

Radioactive sodium was introduced into the hind-leg vein of a dog, and at given intervals thereafter, as previously noted, the secreted juice was measured and blood taken for serum analysis. Samples of the juice and serum were then prepared and tested by the methods described below.

Measurement of radioactivity. Five-tenths or 1.0 cc. samples of pancreatic juice were carefully measured and deposited dropwise upon a blotter measuring 3.5 x 6 cm., which was suspended over an electric hot plate. When thoroughly dry, the blotter was wrapped and sealed in cellophane. Five-tenths cubic centimeter of serum was transferred to blotters and treated in a similar manner. The activities of the blotters were measured with the Geiger-Müller counter, as previously described. Great care was taken to insure uniformity in the mounting of the samples. The sensitivity of the counter was standardized by means of a thorium source before and after each determination. Duplicate samples of serum and pancreatic juice were taken for analysis, and in all cases the results recorded represent the averages of two closely agreeing values.

RESULTS. Observations were made on 9 dogs with pancreatic fistulae; the results obtained were in essential agreement. The excretion of labeled sodium was followed for 22 to 24 hours after the intravenous injection of the radioactive salt. The quantity of sodium chloride injected varied from 40 ml. of an isotonic solution containing a total of 0.04 millicurie of radioactivity to 10 ml. containing 0.96 millicurie. Differences attributable to these variations were not observed. A typical experiment in which 40 ml. of an isotonic solution of sodium chloride containing 0.04 millicurie was injected into a dog weighing 22 kgm. is recorded in figure 1.

The promptness of appearance of administered sodium in the external secretion of the pancreas was noted in all dogs. In 4 animals radioactivity was found in the first sample obtained at the end of 3 minutes. In the

other animals the first samples of pancreatic juice were not taken until intervals of 5, 6 or 10 minutes had elapsed; in all cases labeled sodium was

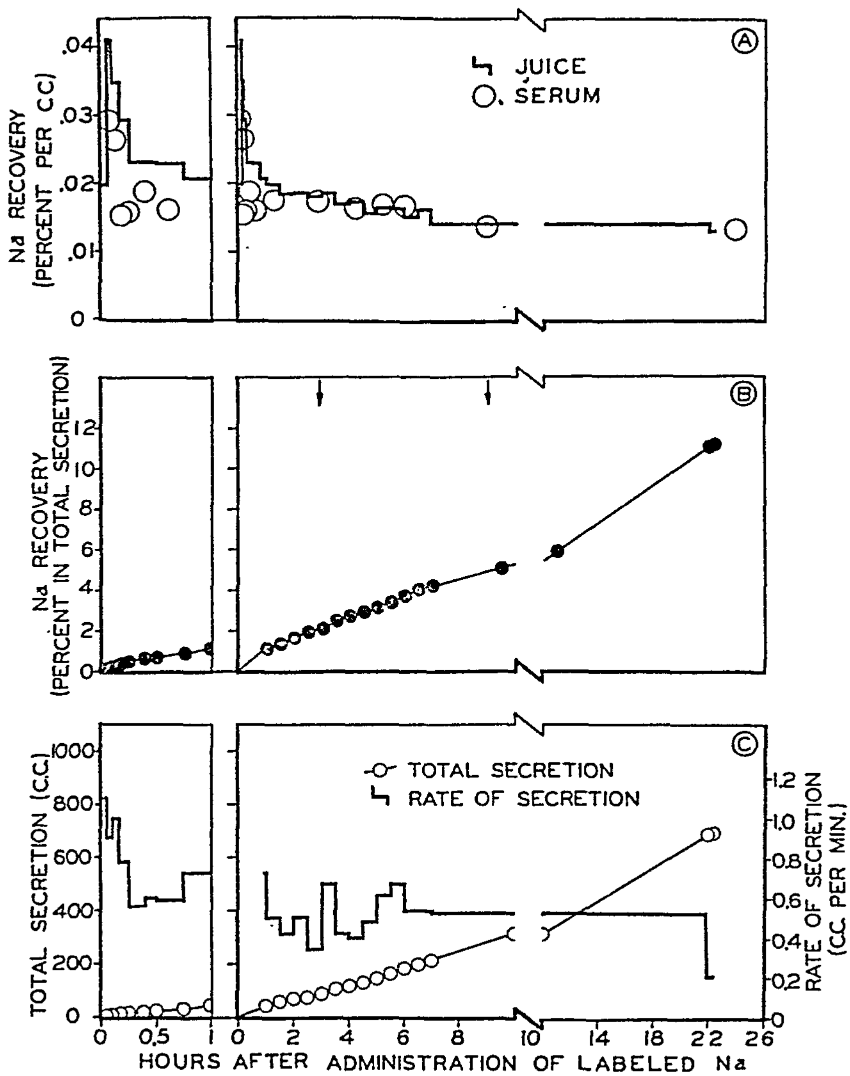

Fig. 1. The secretion of radioactive sodium in pancreatic juice. Dog III, weight 22 kgm. Forty cubic centimeters of 0.9 per cent solution of sodium chloride containing radioactive sodium were injected intravenously at the O interval. In A the concentration of labeled sodium in pancreatic juice and serum is expressed as the percentage of the administered labeled sodium recovered per cubic centimeter. In B the total recovery of labeled sodium is shown and expressed as percentage of the administered labeled sodium. The arrows show the times at which meat was fed to stimulate pancreatic secretion. This animal also received 200 grams of lean meat one hour before the O interval. In C the rate of secretion as well as the total secretion is recorded.

already present. Although the activities of the initial samples were usually low, the highest concentrations of labeled sodium occurred during the first hour. In dog I, labeled sodium was found in increasing amounts in

all samples of pancreatic juice removed during the first 15 minutes after the administration of the radioactive salt, the maximum concentration appearing in the sample obtained between 10 and 15 minutes. In dog II, the highest concentrations were found in the samples obtained after the first 10 minutes, and the content of labeled sodium in the pancreatic juice remained high for the next 50 minutes. The maximum for dog III occurred between 3 and 6 minutes after the injection. The quicker response in this animal is probably the result of the rate of secretion, for it secreted pancreatic juice more rapidly than either dog I or dog II during the early intervals after the administration of labeled sodium.

The similarity in the concentration of labeled sodium in serum and in pancreatic juice is indeed striking. Thus in dog I the sample of serum obtained at the 2-hour interval contained 0.017 per cent of the administered labeled sodium per cubic centimeter, whereas the sample of pancreatic juice removed between the 2 and 2.5-hour interval contained 0.018 per cent per cubic centimeter. At the same time intervals, the serum and pancreatic juice of dog II contained respectively 0.024 and 0.025 per cent of the administered radioactive sodium per cubic centimeter. The sample of pancreatic juice obtained from dog III (fig. 1) between 2 and 2.5 hours contained 0.019 per cent per cubic centimeter, the serum at the 3-hour interval 0.018 per cent of the administered radioactive salt per cubic centimeter. Moreover, the higher concentrations of labeled sodium found during the early intervals parallel the higher concentrations of radioactive sodium present in the serum at this time. It should be noted, however, that in the early periods the concentrations of labeled sodium appear to be somewhat lower in the serum than in the pancreatic secretion. This relation between the levels of labeled sodium in serum and juice is in keeping with previous observations of Gamble and McIver (2) and of others (3, 4), who found that pancreatic juice contains fixed base in concentrations approximating those found in the plasma.

The radioactive sodium used in this investigation was supplied by members of the Radiation Laboratory under the direction of Prof. E. O. Lawrence, to whom our thanks are due.

SUMMARY

1. The elimination of sodium in the external secretion of the pancreas was investigated in dogs provided with pancreatic fistulae with the aid of the radioactive isotope of sodium.

2. Labeled sodium made its appearance in samples of pancreatic juice obtained as early as the first 3 minutes after the intravenous injection of the radioactive salt. Maximum concentrations were found within 15 minutes.

3. Except in the early periods, the concentrations of labeled sodium in the pancreatic juice closely follow those observed in the serum.

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UTERINE RESPIRATION, CYTOCHROME OXIDASE AND COPPER[1]

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The suspicion that cytochrome oxidase may contain copper as its active agent grew with the finding of this metal in tyrosinase and polyphenolase (1, 4, 5, 7). The type of substrate and mode of action characterizing these three enzymes are sufficiently similar (2) to warrant the expectation. To obtain conclusive evidence for the presence of copper in cytochrome oxidase proved difficult because of the insolubility of the oxidase in any medium tested so far. The quantities present are of such concentrations that the suspended state of the enzyme cannot permit even approximate determinations of any reliability.

Obviously such determinations could be made if the enzyme were obtained in soluble form and subsequently concentrated. This has failed and experiments were therefore undertaken to prove the presence of copper in the oxidase by indirect methods.

EXPERIMENTAL. As the source of our cytochrome oxidase we used uteri of rabbits. This tissue contains very little cytochrome but ample oxidase. The oxidase is denatured by water so that unlike beef heart, the ground uterine tissue cannot be washed to remove water soluble material and extraction is made with M/15 phosphate buffer at pH 7.3.

The tissue is ground with sand, the mixture centrifuged and the supernatant used. Measurements of enzyme quantities are determined in Barcroft-Warburg respirometers using p-phenylenediamine as substrate with added excess cytochrome prepared from beef heart. The temperature of the thermostat is 37.50 ± 0.05°C. and the pH of the experimental mixture 7.30.

To begin with, it should be noted that whether the interesting suggestion of Stern (3) with regard to the micelle nature of cytochrome oxidase is fully verified or not, it seems clear that the uterine tissue has only a limited amount of enzyme in the same way as any soluble compound is distributed in any other tissue. Thus, after the oxidase is extracted once

[1] Aided by a grant to Dr. G. Pincus from the National Research Council Committee for Problems of Sex and the Works Progress Administration (Project no. 665-14-3-726).

with phosphate buffer, much tissue remains in the centrifugate but no more enzyme can be obtained from it on further grinding.

Uterine tissue can be made to yield a soluble cytochrome oxidase which, however, is not stable. An enzyme preparation containing 1.5 to 2.0 units per cubic centimeter and appearing homogeneous though slightly opalescent will, on being passed through a bacterial Seitz filter yield a water clear solution. If tested at once the filtrate can be shown to contain up to 0.5 unit per cubic centimeter. It should be stated however that only four such positive results were obtained out of a total of nine trials. The other five yielded no enzyme in the filtrate. Within several hours the activity vanishes and simultaneously a turbidity appears in the solution. It may be assumed that a small fraction of the oxidase consists of sufficiently small particles to form a true solution but these are not stable. They reform the larger particles which do not retain the previous activity of their components. Concentrating and purifying this soluble fraction proved impossible and the extreme dilution precluded any estimation of copper.

Experiments were therefore performed on the effect of copper inhibitors on the oxidation of p-phenylenediamine by copper and by cytochrome oxidase. Several copper inhibiting substances were selected, namely, potassium cyanide, diethyl-dithio carbamate, salicylaldoxime, potassium ferrocyanide, thiourea, hydroxyquinoline and potassium xanthogenate. Each of these was checked in the system-copper sulphate, phosphate buffer, p-phenylenediamine and found to inhibit the oxygen uptake.

It was found that thiourea had a relatively weak inhibiting action on cytochrome oxidase requiring 0.3M solution to reduce the O_2 uptake to 25 per cent, while hydroxyquinoline and potassium xanthogenate, though definitely showing inhibition are however involved in complicating secondary reactions. Results with the first five copper inhibiting substances listed above are recorded in figure 1. With inorganic copper the inhibiting action of these compounds is more or less stoichiometric. This evidence can therefore be taken to indicate that copper is in all likelihood the active metal component in the system cytochrome oxidase and cytochrome.

That it is the cytochrome oxidase that is involved in the inhibition is suggested by the following experiments. To a series of respirometer vessels all containing p-phenylenediamine, buffer, cytochrome and oxidase, any one of the copper inhibitors cited in figure 1, except thiourea, is added in amounts just sufficient to bring about complete inhibition. Fresh cytochrome is then added to one vessel, more enzyme to another, more substrate to a third and buffer to a fourth, the latter to act as control. It is then observed that upon addition of cytochrome no renewed oxygen uptake occurs. Upon addition of new oxidase the uptake is proportional

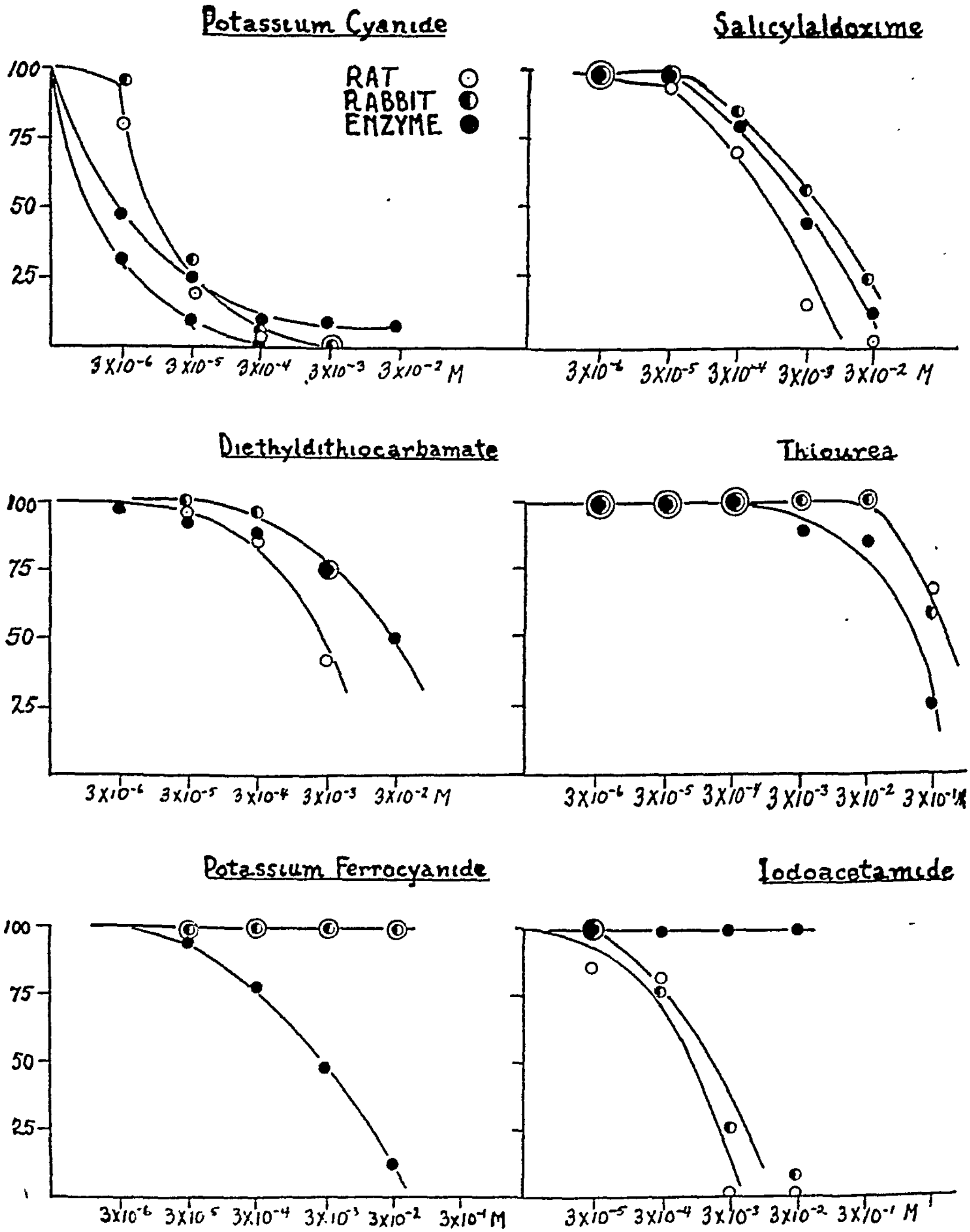

Fig. 1. Curves showing inhibition of cytochrome oxidase extracted from rabbit uteri by five copper inhibitors and failure of iodoacetamide to inhibit it. These curves are to be compared with curves showing inhibition of respiration by rabbit and rat uteri by the same substances. Pregnant and non-pregnant rabbit uteri, endometrium and whole uterus give similar results with regard to inhibition.

Abscissae = concentration of inhibiting substance in experimental vessel.

Ordinates = Q_{O_2} expressed as percentage of normal uptake.

to the amount of enzyme added. It should be noted however that some renewed oxygen uptake occurs as well upon addition of more substrate though such rate is never more than about half the rate obtained after addition of fresh enzyme. This evidence seems to indicate that the copper inhibitors do not affect the cytochrome but primarily the enzyme or enzyme substrate combination.

Experiments were further undertaken to study the effect of these five copper inhibiting compounds upon the total respiration of rabbit and rat uterine tissues. Whole and endometrial tissues of rabbit uteri of non-pregnant and three day pregnant animals were used as described previously (3). The respiration was observed in serum or buffer media. As reported previously no significant differences were observed in these two media. The respiration is calculated on a basis of cubic millimeter of oxygen per hour per miligram dry weight of whole uterus or endometrial tissue. The results are shown in figure 1. It should be noted that the inhibition of various concentrations of diethyl-dithio carbamate could not be completely determined because when concentrations higher than 0.03M are employed there is an evolution of gas and no measurements of oxygen uptake are possible.

Similar experiments were performed with whole rat uteri. These were obtained from 25 to 27 day old animals either normal or injected with pituitary extracts for bioassay. The uteri are placed in respirometer vessels and the rate of oxygen uptake determined per milligram dry weight as described elsewhere (6). Figure 1 indicates that the two types of uteri behave much the same with regard to copper inhibitors.

It should be observed that with potassium ferrocyanide no inhibition of uterine respiration is obtained. This is the only copper inhibitor which fails to inhibit tissue respiration. It may be of interest to recall that many plant and animal tissues do contain an enzyme which oxidizes ferrocyanide to ferricyanide (2). This phenomenon may have some bearing on the exceptional behavior of potassium ferrocyanide. Also in the presence of potassium ferrocyanide the oxygen uptake is slightly higher than in the control.

Discussion. The method employed here in testing the presence of copper in cytochrome oxidase is obviously indirect. The fact that practically all known copper inhibitors inhibit the action of cytochrome oxidase seems to make difficult any other conclusion but that the above oxidase contains copper as its active metal.

A comparison of the inhibition curves of rat and rabbit uteri brings out the close resemblance in the relationship of concentration to inhibition in both enzyme and respiring tissue. In both cyanide seems to inactivate more directly, while both enzyme and tissue show strong buffering power toward the other copper poisons.

It seems reasonable to conclude that cytochrome oxidase is responsible for the total respiration of rabbit and rat uterine tissues. Yet that must not be taken to mean that the respiration of these tissues is controlled exclusively by the cytochrome-cytochrome oxidase system. The curves for the inhibition of uterine respiration by iodoacetamide and its failure to affect the enzyme given in figure 1 prove this point (6, 8). The iodoacetamide is almost as effective an inhibitor of tissue respiration as is cyanide, yet it exerts no influence whatever on the action of the enzyme. This implies that several fundamental reactions may be involved in the control of oxygen uptake by respiring tissue and that such uptake may be blocked correspondingly by different agents.

SUMMARY

1. A water soluble cytochrome oxidase is reported and its transient nature and rapid inactivation described.

2. The inhibition of cytochrome oxidase by substances which inhibit copper catalysis was studied. Evidence is presented which indicates that copper is the active metal of cytochrome oxidase.

3. The rates of oxygen uptake of uteri of rats and of pregnant and non-pregnant rabbits are affected by copper inhibitors in the same manner as is cytochrome oxidase. Hence it is concluded that cytochrome oxidase is directly responsible for the overall respiration of uterine tissues though other substances or reactions may be so linked with it as to be equally determinant.

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THE EFFECT OF ADRENALECTOMY ON THE HISTAMINE CONTENT OF THE TISSUES OF THE RAT

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It has been shown by many investigators that there is a relationship between the adrenal gland and the metabolism of histamine. Following the removal of the adrenal gland in the rat there occurs a decrease in resistance to histamine (Wyman, 1928) which is associated with a marked loss in the ability of the animal to destroy histamine (Rose and Browne, 1938) and a decrease in the histaminase content of lung tissue (Rose and Karady, 1939). It has also been shown that the ability of the adrenalectomized rat to inactivate histamine, and that the decrease in histaminase content of lung tissue following adrenalectomy can be restored to normal by the administration of adequate amounts of adreno-cortical substances (Rose, 1939; Karady, Rose and Browne, 1940).

In view of these findings it was thought that a study of the histamine content of the tissues of the rat before and after adrenalectomy might be of interest.

METHODS. Rats of a hooded strain weighing from 160 to 200 grams were used. The animals were maintained on purina chow and given water to drink. Three groups of animals were used. The first group consisted of 12 animals which served as controls. Since it has previously been shown that the decrease in resistance to histamine in rats following adrenalectomy and maintained on normal saline is fully established by the seventh to eighth day following removal of the adrenal glands (Gottesman and Perla, 1931), a second group of twelve animals was adrenalectomized, maintained on purina and given 0.85 per cent saline to drink for eleven days in order to be certain that the decrease in resistance was fully established. The animals were used on the twelfth day after adrenalectomy. A third group of six animals was adrenalectomized maintained on purina and given 0.85 per cent saline to drink for seven days. The saline was then replaced by water for an additional four days. These animals were quite healthy while they received saline to drink, but they rapidly manifested marked signs of adrenal insufficiency such as weakness, diarrhea and loss of weight when water was substituted. They were killed on the twelfth

[1] Aided by a grant from the Banting Research Foundation.

day after adrenalectomy and the histamine content of the tissues determined. It was noted at the time the tissues were removed that hemorrhagic spots and ulcerations were present in the stomach and small intestine.

Examination of the blood and tissues for histamine was carried out as follows. The animals in each group were anesthetized with ether, the abdomen opened, and a specimen of blood removed from the inferior vena cava. The tissues were then removed and washed free of blood in normal saline. Stomach and small intestine were opened and washed free of their contents. The excess moisture was removed by placing the tissues on large sheets of filter paper. The tissues were then placed in previously weighed flasks containing 10 per cent hydrochloric acid.

Blood histamine was determined by the Code (1937) modification of the Barsoum and Gaddum method (1935). Tissue histamine was determined by a modification of the method of Best and McHenry (1930). The modification consisted of boiling the tissues in the hydrochloric acid over an open flame for one hour instead of placing them in a boiling water bath. It was found that the tissues became fragmented much more easily and that drying "in vacuo" was hastened. There was no difference in the final histamine content of tissues extracted in this way as compared to that of similar tissues prepared by the original method. All assays were carried out on the isolated guinea-pig ileum preparation suspended in Tyrode solution at 38°C. to which atropine was added in a concentration of 1×10^{-6}. Most of the tissue extracts were incubated with a standard histaminase[2] preparation as further test of the nature of the active substance. All values are expressed as histamine base in gamma per cubic centimeter of blood or gamma per gram of tissue.

Results. The histamine content of the blood, stomach, small intestine, kidney, spleen, liver and lung was determined in the first five animals of both the control group and the adrenalectomized animals maintained on saline. It was noted that no marked change occurred in the histamine content of the blood, kidney or spleen of the adrenalectomized animals as compared to that of the controls. The average histamine content of the blood was found to be 0.03 γ/cc., that of kidney 0.42/gram and that of spleen 5.3 γ/gram. A marked increase in concentration, however, was noted in the histamine content of the small intestine and stomach, and a moderate increase in that of the liver and lung. Accordingly the histamine content of only stomach and small intestine was determined in the rest of the animals of both groups and that of the liver and lung in a smaller number. The individual determinations for these latter tissues are given in table 1. It will be noted that there is a marked increase in the histamine

[2] Supplied by the courtesy of the Winthrop Chemical Company and Dr. H. Cave, Montreal, Canada.

content of the tissues of the adrenalectomized animals, that of the stomach being 185 per cent and small intestine 208 per cent of the control values. There is also a moderate increase in the liver and lung to 125 and 120 per cent of the control values respectively.

When water is substituted for saline as in the third group of animals, an even greater increase in the histamine content of the tissues occurs.

TABLE 1

Average histamine content of the stomach, small intestine, lung and liver of control rats, adrenalectomized rats maintained on saline and adrenalectomized on saline for seven days and on water for an additional four days

STOMACH			SMALL INTESTINE			LIVER		LUNG	
Controls	Adrenalectomized and maintained on saline for 11 days	Adrenalectomized and maintained on saline for 7 days and on water for 4 additional days	Controls	Adrenalectomized and maintained on saline for 11 days	Adrenalectomized and maintained on saline for 7 days and on water for 4 additional days	Controls	Adrenalectomized and maintained on saline for 11 days	Controls	Adrenalectomized and maintained on saline for 11 days
9.25	26.4		1.09	6.6	10.7	1.5	2.4	7.8	11.48
9.6	17.3		4.4	8.0	11.8	1.7	2.7	7.3	4.8
15.0	19.5		[illegible]	10.0	9.5	1.2	1.85	8.5	11.3
13.3	20.0		3.0	6.15	10.8	1.0	1.72	5.8	9.7
15.0	24.1		2.6	6.15	8.5	1.3	1.62	6.5	9.5
12.6	31.0	44.4	4.4	5.1	9.5	1.3		9.1	5.8
14.8	22.9	44.6	2.6	4.0		4.3		5.5	9.2
20.0	21.0	43.0	3.1	5.0				6.8	12.7
8.0	20.1	44.0	3.6	7.4					12.7
10.1	26.9	42.8	3.2	8.0					
10.6	25.0	45.2	3.6	7.0					
15.6	31.8		3.1	6.5					
Average. . 12.9	23.9	44.0	3.2	6.6	10.1	1.6	2.04	7.4	9.68
Per cent of normal......	185	334		208	310		125		120

The stomach contains 334 per cent and the small intestine 310 per cent of the control values (see table 1).

As further evidence in favour of the active substance being histamine and not one of the other vaso-dilator substances the extracts were assayed both by the guinea-pig ileum method and by the blood-pressure method on the atropinized cat under Dial anesthesia. As a general rule the tissue extracts were too concentrated for direct assay on the guinea-pig ileum and they were therefore diluted. Examples of assays using both methods for tissues from normal and adrenalectomized animals are shown in figure

1. As a final criterion that the substance involved was histamine, it was found that when the extracts were incubated with a standard histaminase preparation, their potency was destroyed.

Discussion. The increase in histamine content of the stomach and small intestine could be due theoretically to an increased formation of the substance in these tissues, to an increased transfer of histamine to them from other tissues or from the lumen of the gastro-intestinal canal, or to a lessened rate of destruction or excretion of histamine by these organs.

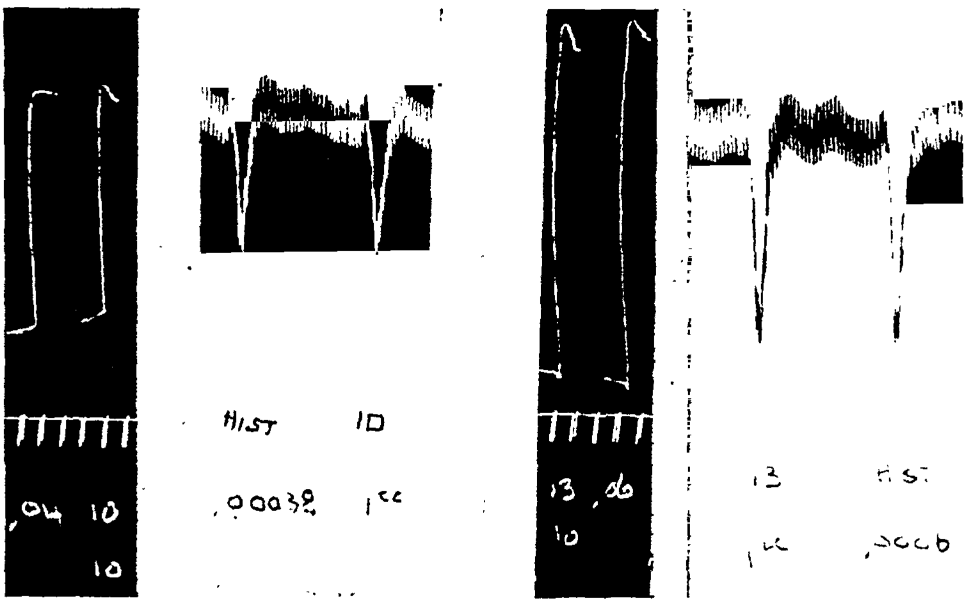

Fig. 1. Assay of extracts of intestine. The assay of extract no. 10, which is of small intestine from a normal rat is shown. At A assayed on the guinea-pig ileum it contains 0.04 y/cc. diluted 1:10. At B assayed by the cat blood pressure 1 cc. original extract contains 0.38 y. In the same figure, the assay of extract no. 13 which is of small intestine from an adrenalectomized rat is shown. Assayed by the guinea-pig method it contains 0.06 y/cc. (shown at C) in a dilution of 1:10. At D it is shown assayed by the cat blood pressure method where 1 cc. original extract contains 0.6 y.

Relatively little is known concerning the mechanism of histamine formation in tissues. Best, Dale, Dudley and Thorpe (1927) were the first to demonstrate its presence in fresh tissue. It is supposedly formed in the gastro-intestinal tract by bacterial action (Koessler, Hanke and Sheppard, 1928) although recent work points to a formation at this site by some other means (Dragstedt, Ramirez de Arellano and Lawton, 1940).

Since adrenalectomy greatly reduces the ability of the tissues of the rat to destroy histamine, it may be that this is responsible for the increase in the histamine content of the tissues observed in the present experiments. The rat stomach does not contain histaminase. It is possible however that

the reduction in histaminase in those tissues which contain it which occurs following adrenalectomy (Karady, Rose and Browne, 1940) is responsible for an increase in the histamine content of the stomach, because more histamine is transferred to it. It could also be due to a decrease in the ability of the stomach either to destroy histamine by some means other than histaminase, or to excrete it into the gastric contents (MacIntosh, 1938).

That ulceration of the gastro-intestinal tract may be produced by the administration of histamine has been demonstrated in many animals (Harde, 1932; Walpole et al., 1940). It may be that similar lesions in animals following adrenalectomy (Selye, 1937a) and in patients with Addison's disease (Maranon, Sara and Arguelles, 1934) may be caused by an increase in the histamine content of the gastro-intestinal tract.

Swingle and his collaborators (1933) suggested that there was a marked similarity between adrenal insufficiency and traumatic shock, and the understanding of the relationship of the adrenal gland to the reaction of the organism to damaging influences such as trauma has been greatly clarified by the work of Selye (1937b). He has suggested that the symptoms of adrenal insufficiency and shock may be due to the liberation of a histamine-like substance from tissues. The results of the experiments described in the present paper tend to support this theory in that there is a marked increase in the histamine content of the viscera in which the ulceration occurs. It has recently been demonstrated (Rose and Browne, 1940), that the blood histamine of patients in various types of shock falls to very low levels during the height of the symptoms and returns to within normal levels as the patient recovers. Since it has been shown that histamine increases in the tissues about an area of inflammation (Rocha e Silva and Bier, 1938), and in the abdominal viscera of the dog following acute burns (Kisima, 1938), it is possible that histamine is transferred from the blood to the gastro-intestinal tract, resulting in a decrease of the blood histamine and an increase in that of the gastro-intestinal tract.

There is thus evidence suggesting that histamine may bear a relationship to the production of the symptoms of adrenal insufficiency and shock and that the metabolism of histamine is influenced by the cortex of the adrenal gland.

CONCLUSIONS

Experiments have been done on the rat to show that there is a marked increase of the histamine content of the gastro-intestinal tract and a lesser increase of the histamine content of the liver and lung following adrenalectomy. The metabolism of histamine and its possible rôle in the production of symptoms in adrenal insufficiency and shock are discussed in relation to these findings.

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THE OXYGEN CONSUMPTION OF SKELETAL MUSCLE FROM ANIMALS DEPRIVED OF VITAMIN E[1]

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It has been abundantly demonstrated that the nutritional dystrophy of voluntary muscle in rats, rabbits and guinea pigs on diets deficient in vitamin E can be prevented and cured by the administration of α-tocopherol (1, 2, 3). The progress of this muscular degeneration is accompanied by a marked decrease in muscle creatine and the appearance of creatine in the urine (4). Other changes in muscle composition and function indicate that the normal course of muscle metabolism is altered in the absence of tocopherol (5, 6). Since this substance is required in very small amounts and is easily oxidized under certain conditions the possible participation of tocopherol in the control of cellular oxidations in muscle cells readily comes to mind.

An increased consumption of oxygen by dystrophic muscle of rabbits was first shown by Victor (7), whose findings were confirmed by Madsen (8) and extended to dystrophic guinea pigs. The total metabolism of such guinea pigs appeared not to be increased (9), whereas that of vitamin E low rats was found to be slightly above that of animals on normal diet (10). It was in rats that the connection of vitamin E with muscle degeneration was first definitely established (11) but no information seems to be available as to altered muscle metabolism when rats are deprived of tocopherol except a note by Drummond (12) to the effect that tissues from E-deficient rats exhibited no diminished oxygen uptake as compared with those of normal animals.

The observations reported in this paper are to the contrary effect: in rats, as in rabbits and guinea pigs, the oxygen consumption of skeletal muscle is above the normal when the animals are maintained on a diet deficient in vitamin E. A few confirmatory measurements were also made on dystrophic rabbit muscle.

EXPERIMENTAL. The control animals were maintained on standard diets,[2] deficient rats were reared from weaning on an E-deficient diet for

[1] From a thesis presented by Irving Friedman to the Faculty of the Graduate College of the State University of Iowa in partial fulfillment of the requirements for the degree of Master of Science.

[2] Purina dog or rabbit chow.

six and thirteen months, and young rabbits were made dystrophic in about three weeks on a synthetic diet which included cellulose, casein, sucrose, starch, salts, cod liver oil, lard and 10 per cent of bakers' yeast.[3]

Oxygen consumption was measured in air in Warburg flasks of the Erlenmeyer type; the temperature of the water-bath was 38°. Thin longitudinal strips of the semi-tendinosus muscle (triplicate samples) were rapidly weighed on a torsion balance (±0.01 gram) and transferred to the Warburg vessels along with phosphate-saline to a final volume of 3 cc., and, in the inner compartment, 0.2 cc. of KOH and filter paper. The vessels were allowed to equilibrate in the water-bath twenty minutes before readings were begun; these were continued for four hours. The tissue was then removed from the flask, quickly rinsed with distilled water, placed in weighing bottles and dried over night at 110°C. for weighing. The results are expressed as Q_{O_2} (cubic millimeters of oxygen per milligram dry weight of tissue per hour). The few creatine determinations were made by the method of Rose, Helmer and Chanutin (13).

The dystrophic rabbit tissue was taken for examination when it became evident that the death of the helpless animals was imminent; they were stunned by a blow on the head. Histological examination was considered unnecessary[4]; macroscopically, the muscles were grayish-white, and appeared to have lost their irritability since they never contracted or twitched as a normal muscle does when it is cut. The data in table 1 show that the oxygen consumption of dystrophic rabbit muscle was about 30 per cent above that of normal rabbit muscle. Qualitatively these results are in agreement with those of Victor (7) and of Madsen (8); quantitatively they are lower both for dystrophic and normal muscles, probably because our measurements were made in air whereas theirs were made in oxygen. This fact may also account for our more rapid decline in oxygen uptake with time; the oxygen uptake was most constant during the second hour.

The rats were injected with sodium pentothal and blood was obtained from the dorsal aorta for use in other studies. The six months old animals were such as could be used for assay of vitamin E; all appeared to be normal in outward respects and had full control of their hind legs. It was striking, therefore, to find (table 2) that the Q_{O_2} during the second hour averaged 2.87 for the muscles of these animals as compared with an average of 1.99 for normal muscle, an increase of more than 40 per cent. The difference in oxygen consumption continued during the remaining two hours. Too few creatine determinations were made to support

[3] Courtesy of Northwestern Yeast Co.

[4] The Department of Pathology, Doctors Brinkhous and Warner, have been very helpful in preparing and interpreting sections of muscle from many similar animals.

any conclusions; the differences here were not as great as in oxygen consumption.

Other older (thirteen months) vitamin E-deficient animals appeared somewhat like those described by Ringsted (14) and more recently by MacKenzie, MacKenzie and McCollum (15). The hind legs were spread out and the posterior part of the abdomen dragged along the ground. The gait was erratic and lacked control. The oxygen consumption (table 2) was only slightly above normal and markedly lower than that of the muscles of the younger deficient animals. Judged by the outstanding degenerative lesions in the dystrophy of older rats (16) the active protoplasmic

TABLE 1

Oxygen consumption and creatine content of normal and dystrophic skeletal muscle of the rabbit

CONDITION	WEIGHT	TISSUE CREATINE	Q_{O_2}* OF MUSCLE			
			1 hour	2 hours	3 hours	4 hours
	grams	*mgm./100 gm.*				
Normal	812		1.61	1.31	1.13	1.02
Normal	810	482	1.76	1.58	1.60	1.35
Normal	850	528	1.71	1.50	1.36	1.30
Average			1.69	1.46	1.33	1.22
Dystrophic	794		2.28	1.97	1.75	1.69
Dystrophic	805		2.29	1.88	1.91	1.96
Dystrophic	808	293	1.98	1.97	1.83	1.83
Average			2.18	1.94	1.83	1.83

* Oxygen consumption in cubic millimeters per milligram of dry weight of tissue per hour.

tissue in such muscles must be greatly reduced; their creatine content was very low.

A few preliminary experiments were performed to determine how quickly α-tocopherol might reduce the oxygen consumption of E-deficient rat muscle to a normal level. Five milligrams of α-tocopherol acetate[5] were fed postabsorptively to five months old female rats whose muscles were examined 24, 72, and 120 hours later. In two of the three series the Q_{O_2} was lower 24 hours after administration of tocopherol than at any other time; in the third series it was lowest after 72 hours. Muscle creatine figures were again too nearly normal to be significant.

Discussion. The increased rate of oxygen consumption by the muscles

[5] Generously supplied by Hoffman LaRoche, Inc.

of rats on E-deficient diets is of the same order as that recently found (measured in oxygen) for the diaphragm of rats on diets deficient in riboflavin and in the other heat-stable components of the vitamin B complex (17); no histological changes could be detected in the diaphragm.

TABLE 2

Oxygen consumption and creatine content of semi-tendinosus muscle

SEX	WEIGHT	MUSCLE CREATINE	Q_{O_2} OF MUSCLE			
			1 hour	2 hours	3 hours	4 hours
Normal rats						
	grams	*mgm./100 gm.*				
♂	372		1.98	1.89	1.71	1.63
♂	308		2.10	1.94	1.77	1.58
♂	196		2.02	1.85	1.62	1.52
♀	260		2.18	1.84	1.73	1.66
♂	230	401	2.15	2.17	2.05	1.89
♀	298	436	2.71	2.25	1.94	1.91
Average....	277		2.19	1.99	1.80	1.70
Vitamin E deficient rats (five months old)						
♀	216		3.81	2.97	2.51	2.32
♀	200		3.77	3.17	2.82	2.57
♀	204		2.86	2.51	2.00	2.16
♀	206		3.43	3.17	2.59	2.27
♀	216		2.84	2.43	2.12	1.84
♀	208		2.79	2.38	2.19	2.02
♀	204		2.93	2.57	2.29	2.07
♀	230	393	3.39	2.89	2.43	2.00
♀	220	393	3.27	2.71	2.39	2.19
Average....	212		3.23	2.87	2.42	2.16
Severely paralyzed vitamin E deficient rats (thirteen months old)						
♀	164	302	2.79	2.24	1.94	1.84
♀		331	2.52	2.17	1.88	1.71
♀	200	321	2.44	2.50	2.14	2.04
♀	220		2.49	2.16	2.04	1.75
♂	260	300	2.36	2.00	1.77	1.61
Average....	211	314	2.52	2.17	2.03	1.79

Similarly, in vitamin E deficient animals, Knowlton and Hines (6) found no gross symptoms of dystrophy and only minor histological changes in the muscles, but the gastrocnemius showed decreased maximal contractile power, decreased creatine and increased chloride concentration. A lack of heat-stable members of the vitamin B complex can not be associated

with the increased oxygen uptake in our experiments since the E-deficient diet contained untreated casein and 8 per cent of yeast.

Verzar (18) concluded that vitamin E exerts its effect directly on muscle since creatine excretion in the urine of dystrophic rats decreased immediately upon feeding large doses (200 mgm.) of α-tocopherol and again increased as soon as the administration of α-tocopherol was stopped. The possible rôle of tocopherol in cellular oxidation has been mentioned by several workers (19); the physiological processes concerned appear to be under some nervous control (20), perhaps of a trophic nature.

The interesting suggestion has been made (21) that the existence in tissues of a series of biocatalysts for oxidation accomplishes gradual degradation of a metabolite such that its total energy is released stepwise rather than completely at a single bound. With this figure in mind one might postulate that in the absence of tocopherol one of the intermediate and delaying steps drops out and that oxidation therefore proceeds the more rapidly; in vitro, under some conditions, α-tocopherol is an antioxidant. The nature of the metabolites concerned in this accelerated muscular oxidation and the possible agencies associated with tocopherol are under investigation.

SUMMARY

1. The oxygen consumption of the semi-tendinosus muscle of six months old rats, reared from weaning on a diet deficient in tocopherol, was 40 per cent above that of normally fed rats.

2. Older (thirteen months) animals that were severely paralyzed through lack of tocopherol showed a much smaller elevation of the oxygen consumption.

3. In confirmation of observations of others, the oxygen consumption of the semi-tendinosus muscle of rabbits, made dystrophic by a diet deficient in tocopherol, was higher than that of normal rabbits.

4. The possible significance of these observations is briefly discussed.

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RENAL RESPONSE TO REPEATED ADMINISTRATION OF POST-PITUITARY EXTRACT

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Howell in 1898 first showed that the rise in blood pressure obtained after the injection of post-pituitary extract became less noticeable and finally disappeared on repetition of the dose of the extract. He was also unable to maintain an increased blood pressure by the injection of rapidly successive doses of the extract, as were later workers in the field (see Van Dyke, 1936, 1939). In 1914 Herring reported that a second dose of post-pituitary extract, administered after the renal effect of the first dose had passed off, was again active, although the kidney did not expand again to the same extent. Geiling (1926) also noted the fact that diuresis (in anesthetized animals, presumably) occurred after repeated injection of the extract, even though the effect on blood pressure became small or even gave way to a depressor effect. These occasional experiments were performed, however, at a time when there was still much confusion regarding the primary effect and mechanism of action of the antidiuretic hormone; and the question of renal tolerance to repeated injections of post-pituitary extract has apparently not been fully investigated in controlled experiments on unanesthetized animals. The experiments reported herein have been designed to test the effects of successive doses of post-pituitary extracts on animals under physiological conditions.

METHODS. Male white rats, weighing about 200 grams, were used, and 18 animals comprised each experimental group. Food was withheld for 12 hours before each experiment, though water was freely accessible during this time. The method of urine collection has been previously described (Silvette, 1940).

It had previously been determined that fasted white rats allowed water ad libitum drank on the average 7 cc. per 100 grams of body weight and excreted between 5 and 6 cc. of urine per day (Corey, Silvette and Britton, 1939). In order to approximate the normal free intake of water, the experimental animals were therefore given intraperitoneal injections of 2 cc. per 100 grams of body weight every 8 hours in the first group of experiments, and 0.5 cc. every 2 hours in the second group. The urine volumes were recorded at the end of each 8-hour or 2-hour subdivision.

Besides control animals receiving intraperitoneal injections of distilled water alone, other animals were given injections of water plus U.S.P. XI posterior-pituitary solution[1] and of pitressin. The dosage employed was maximal, being 1 U.S.P. unit of the former and 2 pressor units of the latter (i.e., 0.1 cc. of each extract) per 100 grams of body weight every 8 hours in the first group, and 0.5 U.S.P. unit every 2 hours in the second. In the earlier experiments injections of the extract were made subcutaneously at the same time the intraperitoneal injections of water were made; but it later became apparent that combining the pituitary extract and water into a single intraperitoneal injection gave similiar results to administration by divided routes, and the simplified procedure was then adopted.

Results. The results have been analyzed statistically and are shown in tables 1 and 2 as averages of 18 animals $\pm$ the probable error of the average. Comparison of results obtained by the use of the U.S.P. pituitary solution, containing both pressor and oxytocic fractions (table 1, B), with those given by the pressor substance alone (table 1, C), indicated that the essential antidiuretic action shown in these experiments was not modified in any way by the oxytocic substance present in the whole lobe extract. It also appeared that unmodified post-pituitary solution produced as marked an antidiuretic effect, cubic centimeter for cubic centimeter, as Pitressin alone, although the pressor activity of the latter is said to be double that of the U.S.P. pituitary solution (Sollmann, 1937).

It will be noted that the initial dose of either of the pituitary extracts inhibited urine flow (table 1, B and C), compared with the control output (table 1, A), and that this same degree of inhibition was uniformly maintained by successive injections throughout a 48-hour metabolic period. That there was no escape from pituitary antidiuresis during this time was shown by the fact that when the extract injections were stopped after the first 24 hours, the antidiuresis also ceased, and the urine output rose to the control level and finally above it (table 1, D).

In a second group of animals the above experiments were repeated, injecting pituitary extract and recording urine output every 2 hours instead of every 8 hours (table 2). In 8 of the 18 pituitary-injected animals the injections of extract were continued for 26 hours and then stopped, whereupon the urine output rapidly rose to the control level. It will be noted that the results obtained using either 8-hour or 2-hour injection intervals were practically identical, indicating a continuous antidiuretic effect (table 1, D compared with table 2, B).

Discussion. The results of the experiments described herein, while interesting in themselves, afford an opportunity for a theoretical discussion

[1] Posterior pituitary solution Squibb, kindly furnished by Dr. John F. Anderson of E. R. Squibb and Sons.

of the mechanism of post-pituitary action which should point the way to future experimentation.

TABLE 1

Effect of repeated doses of post-pituitary extract on urine output

SERIES	FLUID INJECTED*	CUMULATIVE URINE OUTPUT IN CUBIC CENTIMETERS PER 100 GRAMS B. W. AT END OF					
		8 hours	16 hours	24 hours	32 hours	40 hours	48 hours
A	Distilled water	1.4 ±0.08	3.6 ±0.13	4.9 ±0.13	6.3 ±0.16	7.7 ±0.18	8.6 ±0.17
B	Water + 0.1 cc. pituitary extract, U.S.P.	0.8 ±0.09	1.9 ±0.12	2.9 ±0.14	4.0 ±0.16	5.1 ±0.20	5.9 ±0.18
C	Water + 0.1 cc. pitressin	0.9 ±0.04	2.1 ±0.08	3.0 ±0.14	4.1 ±0.16	5.4 ±0.17	6.8 ±0.20
D	Water + 0.1 cc. pituitary extract for 3 inj.; then water alone for 3 inj.	1.2 ±0.07	2.1 ±0.11	3.2 ±0.13	6.6 ±0.21	8.7 ±0.22	9.9 ±0.23

* Two cubic centimeters per 100 grams' body weight every 8 hours. For further details, see text.

TABLE 2

Effect of bi-hourly doses of post-pituitary extract on urine volume

SERIES	FLUID INJECTED*	CUMULATIVE URINE OUTPUT IN CUBIC CENTIMETERS PER 100 GRAMS B. W. AT END OF															
		2 hours	4 hours	6 hours	8 hours	10 hours	12 hours	14 hours	16 hours	18 hours	20 hours	22 hours	24 hours	26 hours	28 hours	30 hours	32 hours
A	Distilled water	0.5 ±0.10	0.9 ±0.07	1.2 ±0.09	1.8 ±0.10	2.1 ±0.14	2.6 ±0.14	2.9 ±0.16	3.6 ±0.13				4.9 ±0.13				6.3 ±0.16
B	Water + 0.05 cc. pituitary extract, U.S.P.	0.3 ±0.03	0.7 ±0.05	1.0 ±0.05	1.1 ±0.04	1.3 ±0.06	1.4 ±0.06	1.6 ±0.07	1.7 ±0.07	2.0	2.3	2.5	2.8	3.0†	3.6	4.4	6.0

* Five-tenths cubic centimeter per 100 grams' body weight every 2 hours. For further details, see text.

† Injection of pituitary extract stopped and distilled water continued alone.

The effect of repeated injections of post-pituitary solution in steadily inhibiting urine flow is in marked contrast to the inability of the extract to maintain an increased blood pressure even on practically continuous injection. It is generally assumed that both pressor and antidiuretic actions

of posterior lobe extracts are due to a single hormone (Van Dyke, 1939), which has a stimulating action on both "pressor" and "antidiuretic" receptor mechanisms, causing the smooth musculature of certain blood vessels to contract, and the tubule cells of the kidney to reabsorb water at a higher rate. It seems probable that the hormone produces its typical effect only as long as it remains in contact, in sufficient concentration, with the receptor mechanisms. Thus, after subcutaneous or intramuscular injection of the extract, there is generally no pressor response (Van Dyke, 1936), apparently because absorption is so slow that at no time are the blood-vessel walls bathed in a sufficiently high concentration of the hormone to cause contraction of the musculature. The tubule cells seem to be affected by a much lower concentration, for subcutaneous injection in the present experiments produced typical antidiuretic effects, while presumably no simultaneous increase in the blood pressure took place.

The question of tolerance would seem to be bound up with the difference in sensitivity. The smooth musculature of the blood vessels appears to be sensitive only to relatively high concentrations of the hormone, and regains its sensitivity very slowly after once being acted on by an effective concentration. The renal tubule cells, on the other hand, would seem to retain their sensitivity indefinitely unimpaired. That the tolerance acquired by the blood vessels should not be shared by the tubule cells is physiologically necessary according to the theory that water balance is maintained by the antagonism between an antidiuretic pituitary hormone and a diuretic cortico-adrenal hormone, the one operating to increase and the other to decrease tubular reabsorption of water (Silvette and Britton, 1938). In order for such an antagonism to be effective, the tubule cells would have to be constantly sensitive to changing amounts of the two hormones.

SUMMARY

The inhibitory effect on urine flow of the antidiuretic hormone of the post-pituitary is maintained, on repeated injections either 2 or 8 hours apart, for at least 48 hours, and during this time no tolerance for the extract is developed. When pituitary extract administration is stopped, the antidiuresis promptly subsides and the urine output reaches the control level. The physiological significance of these observations is discussed.

Post-pituitary solution (U.S.P. XI) and Pitressin have similar effects on urine output, indicating that the oxytocic fraction present in the U.S.P. extract is without influence on the results.

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THE MECHANICS OF GASTRIC EVACUATION[1]

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According to the laws of hydraulics, material in the gut tends to move from a region of higher intralumen pressure to one of lower pressure. The rate of translocation of material from one cavity to another may be expressed as a function of this differential pressure and the resistance between the two regions: Rate of flow $= K \dfrac{\text{Pressure A} - \text{Pressure B}}{\text{Resistance to flow}}$. Accordingly, the relation of pressures in the pyloric antrum and duodenal bulb plays an important rôle in the process of gastric evacuation.

Although antral pressure in excess of bulbar pressure is essential for gastric evacuation, expulsion of gastric contents will not necessarily obtain whenever this gradient develops. The pressure, P, developed within a cavity is related to the time-rate of volume change in the cavity, dv/dt, and, R, the resistance to the escape of contents, as shown by the formula $P = (dv/dt)R$. Thus, an increase in resistance interferes with propulsion but favors the development of pressure.

Several of the factors involved in gastric evacuation were investigated as follows: The pressures in the antrum and bulb were accurately measured by the method of Brody, Werle, Meschan and Quigley (1). We employed trained dogs provided with permanent metal cannulae giving access to the stomach and duodenum. Through these cannulae we introduced rubber tubes carrying open plastic recording tips. The open ends of these were arranged to lie 3 to 4 mm. at either side of the pyloric sphincter (fig. 1). The tips contained lead foil inserts to permit roentgenological localization. Pressures from the antrum and bulb were recorded on the photokymograph by optical capsules. Barium sulfate incorporated with mush was fed to the animals and simultaneously with the pressure studies, fluoroscopic determinations were made of the time of 1, antral peristaltic waves from their origin near the incisura angularis to their termination at the sphincter (multiple waves, when present, were recorded separately, fig. 2, B);

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2, passage of material through the sphincter; 3, bulbar filling, and 4, bulbar evacuation. Several observers were inclosed in a light proof cabinet to observe the fluorescent screen and each observer recorded the phenomenon assigned by pressing a key. A mirror was thus exposed which projected a beam of light to the photokymograph where antral and bulbar pressures were being recorded. The time of shortening of the sphincter diameter from the beginning of contraction to the beginning of relaxation was like-

Fig. 1. Reproduction of a radiograph taken from a dog immediately after a barium meal was fed. The small oval shadows indicate the position of shot attached to the serosa at either side of the pyloric sphincter. The cylindrical object below the shot marks the antral recording tip; the bulbar tip is above.

wise recorded by observing the fluoroscopic shadow of lead shot attached to the serosa at either side of the sphincter (fig. 1). The personal factors in observation were minimized by frequent rotation of the experimenter's duties. Registration of the time of pressure changes is entirely accurate but the time relations of the fluoroscopic observations is less satisfactory. A lag in the registration time of beginning and termination of these events is due partly to the reaction time of the recorder (ca 0.20 sec.), but more significant, especially in the registration of shot movements, is the fact

that certain changes in activity of small magnitude are not promptly appreciated.

Indirect information regarding pyloric sphincter activity can be derived from an examination of antral and bulbar pressure. The sphincter probably is firmly closed and resistance to flow is great when antral and bulbar pressures differ markedly, but when the pressures approach each other, communication between the two cavities may or may not be present.

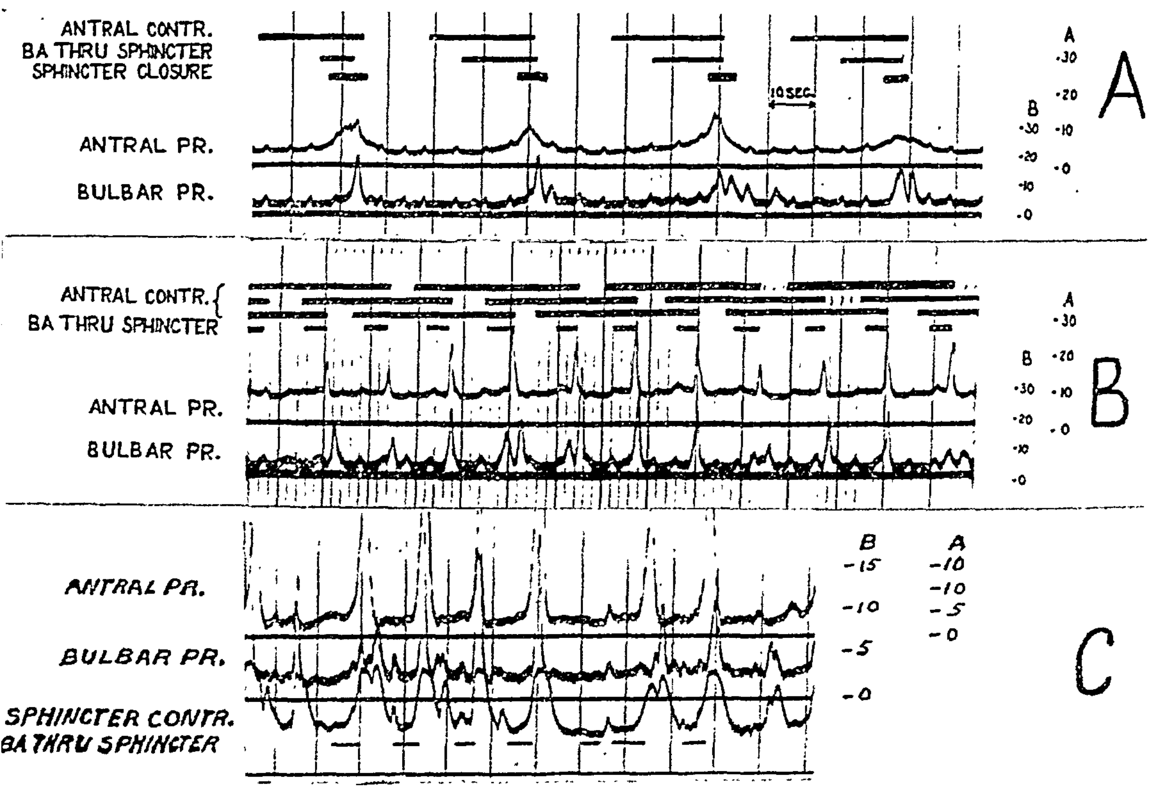

Fig. 2. Interrelation of the events occurring during the gastric evacuation of a meal of corn meal mush and $BaSO_4$. A. When single peristaltic waves pass over the antrum, recorded by a single set of signal bars. Study of sphincter behavior by observing shot movements. B. When three antral waves pass progressively over the antrum, indicated by three sets of signal bars. C. Study of sphincter behavior by optical registration from a sphincter balloon.

When the sphincter is relaxed, the pressures in the two regions should be similar though not necessarily identical, for sufficient resistance in the sphincter region may be anticipated from the anatomical conformation, etc., to explain moderate differences in the pressures in the antrum and bulb.

Measurements of the external diameter at the sphincter are obtained from observations on the shot position, but information regarding the internal diameter of the sphincter would be preferable. The two distances

will be related, but since the sphincter muscle thickens during contraction, the degree of sphincter closure will be greater than is indicated by the decrease in the external diameter. It would be advantageous to have observations of the tone and contractions of the sphincter musculature. The sphincter diameter is the sum of this motor activity plus those factors tending to produce passive sphincter distention. Usually in our records these forces can be differentiated. For example, in figure 3, IV, there are no significant forces tending to produce passive distention, therefore the widely separated shot indicate sphincter relaxation. In this report we

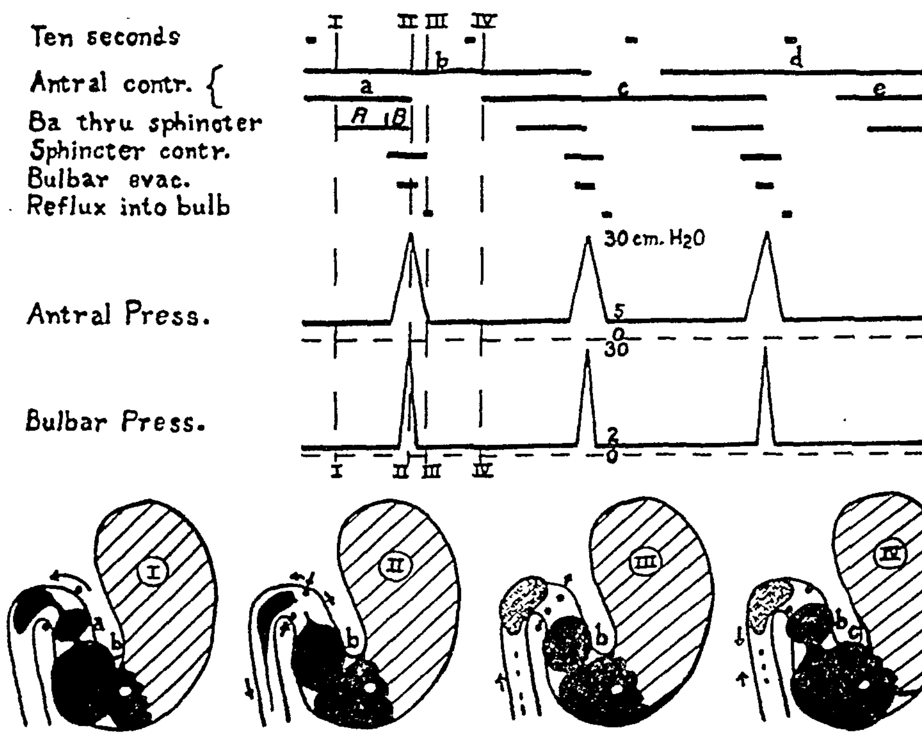

Fig. 3. Evacuation-pressure cycle schematized in four phases. The sketches depict the events at each phase of the cycle at the time indicated by the vertical line designated by the corresponding Roman numeral.

have used this method when feasible to state the type of sphincter activity. We are positive the sphincter is patent only when it is both relaxed and passively dilated, as in figure 3, I, and therefore have applied the term "open" only when this condition obtains. We obtained additional information regarding sphincter activity from a 5 x 7 mm. crescent-shaped balloon anchored in the sphincter lumen which registered by means of an optical manometer. Apparently the balloon was unaffected by pressure variations in the antrum and bulb for the records obtained from these regions differed in time, character and magnitude of change. The time of beginning and cessation of sphincter contraction and also the type of

change in sphincter activity were recorded rather accurately, but exact pressure values were not obtained by this method.

After feeding 150 to 1000 grams of strained mush (cooked corn meal and meat), mixed with 45 to 90 grams $BaSO_4$, records were obtained (fig. 2, A, B, C) which characteristically showed cyclic pressure changes in which maxima of about the same magnitude occurred simultaneously in both the antrum and bulb. During each phasic wave the antral pressure was temporarily in excess, for the pressure rise began earlier in the antrum and terminated later than in the bulb. Also, the basal pressures occurring between phasic changes showed a gradient, for antral pressures were 5 to 8 cm. of water and bulbar pressures 2 to 4 cm. Thus, during each cycle, a pressure gradient which could produce gastric evacuation was present during period A, the interval in which pressures in both antrum and bulb were at the basal level; period B, the first portion of the antral phasic wave; period C, the last portion of the antral phasic wave. Evacuation occurred only during periods A and B but not during C.

Although activity in the pyloric region did not follow a rigid behavior pattern, the following sequence of events occurred most frequently. It was observed fluoroscopically that as a peristaltic wave traversed the antrum it propelled gastric contents towards the sphincter. While a single wave involved the proximal antrum there was no escape of material through the sphincter and the pressure in the prepyloric region remained at the basal level. When the wave reached the distal half of the antrum, evacuation began (evacuation period A). The shot were widely separated, a stream of material passed through the sphincter and frequently some returned to the body of the stomach. The head of pressure supplied by the antrum, although it remained at basal level, was evidently adequate for this phase of the evacuation since the resistance to flow through the sphincter and into the bulb was low.

When the peristaltic wave reached the lower antrum, approximation of the shot began, thus indicating contraction of the sphincter. Observations of the rate of bulbar filling failed to demonstrate that this necessarily decreased the evacuation rate. As the sphincter gradually contracted it provided the increased resistance (R in the formula $P = dv/dtR$) necessary for the antral phasic wave. The peristaltic wave progressively cut deeper and moved to regions of smaller diameter, thus increasing R and dv/dt. Therefore antral pressure increased sufficiently to continue the expulsion of contents (evacuation period B) in spite of the contracting sphincter. Bulbar pressure remained at the basal level. As the antral pressure reached higher levels and the sphincter closed completely the very small amount of material remaining in the distal antrum was forced proximally. The antral pressure fell after the peak of the phasic wave

as the distal antrum began to relax. A decrease in resistance distally was not involved for the sphincter usually had not started to relax when antral pressure began to return to the basal level.

Gastric evacuation ceased because 1, the sphincter was closed; 2, the elevated bulbar pressure provided additional resistance; 3, the prepyloric region was essentially empty. A sphincter contraction always accompanied an antral or bulbar phasic wave regardless of whether, as usually happened, the antral and bulbar waves occurred together, or were independent of each other. Termination of antral evacuation usually coincided with the beginning of the plateau of the sphincter wave and also approximately with the middle of the ascending limb of the bulbar wave. Since the several factors already mentioned occur simultaneously it is impossible to state positively that a sphincter contraction acting alone will normally stop evacuation.

Basal pressure persisted in the bulb as it filled during evacuation period A and the first part of period B, then, with the onset of bulbar contraction, the pressure rose sharply to produce the bulbar phasic wave. Since the sphincter at this time was closing and antral pressure was high, regurgitation into the stomach was prevented. Cole (2), as well as Meschan and Quigley (3), obtained evidence that the sphincter serves the important function of interfering with bulbar regurgitation. Apparently, considerable resistance was encountered to movement down the duodenum (e. g., the material passed distally in a narrow stream). This resistance made it possible for bulbar pressure to rise, i.e., in the formula $P = dv/dtR$, R was great. Also, the rapid bulbar contraction was significant, since it augmented the factor dv/dt. The pressure rose sharply to reach a maximum approximately coincidentally with the peak of antral pressure. The notched bulbar pressure waves sometimes observed usually resulted from repeated bulbar contractions for associated expulsion of bulbar contents, as well as the contractions themselves, were visualized. Periodic changes in the resistance to the escape of duodenal contents could give a similar pressure change. Bulbar pressure fell sharply as most of the bulbar contents was expelled and the bulb relaxed. Relaxation of the sphincter was not essential for this fall, for spreading of the shot usually began after the bulbar pressure had returned to the basal level.

During the later half of the interval required to evacuate a meal, a portion of dilute material frequently returned from the second part of the duodenum to the bulb immediately after the bulbar relaxation (fig. 3, III, IV). This phenomenon is similar to the to-and-fro movement of material in the vertical duodenum—"dancing particles" which have been noted by Barclay (4), and to the regurgitation of barium in the same region reported by Todd (5). Apparently the regurgitation noted by us was

associated with considerable relaxation of the bulb for the bulbar shadow at this time was larger though fainter than during the antral evacuation period.

Several types of deviation from the typical pattern of evacuation described above were noted. When a peristaltic wave swept over the antrum, any fraction of the material initially propelled by the wave might return to the body of the stomach. Usually, most of the material was evacuated, but occasionally, as noted by Cannon (6, p. 96), peristaltic waves passed over the antrum for some time without producing evacuation. If the antral waves were infrequent, shallow, and tended to die out before reaching the sphincter, this failure to evacuate was readily explained. When a vigorous peristaltic wave died out near the sphincter, evacuation period A frequently was present without period B or the corresponding antral phasic wave. Period B might also be absent if the sphincter and bulb failed to develop resistance to the expulsion of antral contents. There were also occasions when an apparently vigorous peristaltic wave swept over the entire antrum, a typical antral phasic wave was recorded, the sphincter was relaxed, a pressure gradient from antrum to bulb was present but evacuation was absent. Usually the food was propelled $\frac{2}{3}$ the length of the antrum by a vigorous wave. The food then returned to the body of the stomach while the wave continued and produced a typical phasic wave. Evidently a further factor which has not been studied in this investigation is essential for evacuation.

Study of the shot movement and the sphincter balloon records showed the contraction wave of the sphincter involved 15 to 50 per cent of each evacuation cycle. Fluoroscopic observations indicated that the sphincter effectively stopped evacuation during only $\frac{1}{2}$ to $\frac{1}{3}$ of its contraction period. Evacuation failed to occur throughout the entire interval during which the sphincter was relaxed, e.g., at figure 3, IV, but it was also noted that at this time the prepyloric region was empty. Gastric evacuation occurred only while the peristaltic wave involved the terminal antrum. When a second or third antral wave started before the first died out, emptying occurred only during the period of maximal overlapping of waves (fig. 2B).

The material discharged from the antrum usually lodged temporarily in the bulb, but when evacuation occurred very rapidly the stream might pass directly down the duodenum before the onset of the typical bulbar contraction. In the latter event, bulbar evacuation began 1 to 2 seconds after the onset of antral emptying and usually persisted for several seconds after its termination.

The peaks of antral and phasic pressure waves usually coincided, but slight variations in which either peak might precede were common. More rarely, an interval of several seconds elapsed between peaks and occasion-

ally an independent antral or bulbar wave developed. A contraction of the sphincter apparently was associated with each of these types of pressure waves. Evacuation usually occurred with each modification of the normal pattern, but in addition to the exceptions already considered, the isolated bulbar wave produced neither gastric evacuation nor bulbar regurgitation. With the onset of evacuation period A the rate of discharge rapidly rose to a plateau which was maintained during this period when the pressure gradient was 3 to 5 cm. of water. Of the total quantity discharged in the cycle, $\frac{1}{2}$ to $\frac{2}{3}$ is expelled during this period. As period A was superseded by period B the gradient gradually rose to approximately 25 cm., but the evacuation rate tended to be maintained, apparently because of the balance between the augmenting antral pressure and the increasing resistance to flow offered by the sphincter. The effective pressure (pressure gradient—resistance) probably remained relatively constant until evacuation terminated abruptly usually with completion of the sphincter closure and the development of the bulbar phasic wave.

At the onset of gastric evacuation the differential pressure relation between antrum and bulb usually remained unchanged, but occasionally it slightly rose or fell. Several factors were integrated with those previously considered to determine the pressure at this moment. During evacuation, material was flowing away from the antral recording tip and toward the bulbar tip. According to the Pitot principle this should lower the recorded pressure gradient. This influence should be slight, for in the process of evacuation the dynamics of flow were feeble. Also, with the onset of emptying, a portion of the energy derived from the contraction which might have been converted into pressure, appeared as energy of flow. Thus the antral pressure showed little tendency to rise. The energy expended by the antrum during the evacuation interval probably was greater than during the preceding portion of the cycle.

We prepared differential pressure records by subtracting bulbar pressures from antral pressures. Such records displayed too much individual variation to be of much significance in explaining the mechanism of evacuation. Since antral basal pressure uniformly exceeded bulbar, the contour of the differential records varied chiefly with the magnitude and time relations of the phasic pressure changes. These time relations normally showed considerable variation and in addition the pressures recorded were greatly influenced by the distance between the recording tips. The most striking changes in a differential pressure record were usually observed during the less important portion of the evacuation cycle. A differential pressure record resembling the three phase cycle of Thomas (7) could occasionally be obtained, but it appeared to be a fortuitous rather than a characteristic result.

SUMMARY

Simultaneous optical registration of intralumen pressures of the pyloric antrum and duodenal bulb of unanesthetized dogs combined with fluoroscopic observations of this region was employed in studying gastric evacuation. In each evacuation cycle the first period of evacuation (period A) was associated with a moderate basal pressure gradient from antrum to bulb. During this interval the pyloric sphincter and duodenal bulb offered very little resistance to expulsion. Since the antral pressure remained low, the energy of the antral contraction occurring at that time probably was chiefly transformed into propulsive force. As the accumulation of material in the duodenum and the contraction of the sphincter increased the resistance distally, the advancing antral peristaltic wave elevated the antral pressure and the antral phasic pressure wave was produced. Gastric evacuation persisted (period B) under the augmented pressure head until terminated by several factors, among which was the completely contracted sphincter. Bulbar contraction occurred at this time and caused bulbar emptying, but regurgitation was prevented by the contracted sphincter.

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RECOVERY OF FATIGUED MUSCLE FOLLOWING INTRAVENOUS INJECTION OF POTASSIUM CHLORIDE[1]

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A temporary increase in the height of contraction of normal skeletal muscle is known to follow the close arterial injection of potassium chloride (2, 3, 4, 11). Baetjer observed this increase in fully curarized muscle (2), and concluded that it was therefore due to a direct effect of potassium on the muscle fibers. Since potassium has some anticurarizing action on neuromuscular transmission (3, 4, 11), this conclusion may not be warranted on the basis of Baetjer's experiments alone. However, the demonstration by Brown and von Euler (3) that the increase may be elicited in previously denervated muscle proves conclusively that it must at least in part be due to a direct effect on muscle, in addition to any facilitation of neuromuscular transmission.

Wilson and Wright (11) failed to elicit any appreciable increase in the height of contraction following the intra-arterial injection of potassium in fatigued muscle. Winkler, Hoff and Smith (13), however, have observed that the height of contraction of skeletal muscle is well maintained during the course of the intravenous injection of potassium chloride; late in the course of the injection the height of contraction may even be greater than at the beginning. This is remarkable since fatigue alone would cause a decrease in the height of contraction during the course of the experiment. It accordingly seems probable that potassium injected intravenously does affect fatigued muscle. The experiments to be described deal with the effects of such injections on fatigued muscle.

METHODS. Cats deeply anesthetized with nembutal were used throughout. In the two experiments with neuromuscular transmission the left quadriceps, after being isolated by appropriate nerve and tendon section, was arranged to pull against a torsion wire myograph writing by means of a straw on a smoked drum. The nerve was stimulated by supramaximal

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break shocks delivered to the motor nerve every two seconds from a thyratron stimulator. The resulting twitches were recorded on a slowly revolving drum. The circulation to the muscle was intact, and the muscle was kept warm and moist by means of a cellophane shield and a constant drip of warm Ringer's solution. After a period of stimulation sufficient to cause marked reduction in the height of the twitches, an isotonic solution of potassium chloride was introduced through a cannula into a leg vein at the rate of 2 to 5 cc. per minute. Injection was not continuous, however, but was interrupted for several minutes between each 5 cc. aliquot

TABLE 1

Potassium chloride solution (0.154 M) injected intravenously into cats

EXPERIMENT	TYPE OF STIMULATION	PERIOD OF FATIGUE	TENSION* DEVELOPED AFTER FATIGUE (PER CENT OF INITIAL VALUE)	TENSION* DEVELOPED AFTER KCl INJECTION (PER CENT OF INITIAL VALUE)	VOLUME OF KCl SOLUTION INJECTED
		min.			*cc.*
1	Neuromuscular	69	63	99	45
2	Neuromuscular	325	75	92	20
3†	Direct muscular	0		138	85
4†	Direct muscular	0		100	25
5	Direct muscular	240	46	64	15
6	Direct muscular	95	75	85	55
7	Direct muscular	28	82	164	25
8	Direct muscular	31	75	127	35
9	Direct muscular	15	68	110	60
10	Direct muscular	95	48	77	60
11‡ (a)	Direct muscular	85	46	91	25
(b)		78	45	93	30
12‡ (a)	Direct muscular	45	49	81	25
(b)		60	61	68	25

* The initial tension developed is taken as 100 in each instance.

† No fatigue; control.

‡ Two periods of fatigue and of injection.

of solution. Injections of 5 cc. amounts were repeated in this manner until death of the animal from the toxic effects of potassium on the heart (12).

In the great majority of the experiments the sciatic nerve had been sectioned 4 to 7 days previously. The combined gastrocnemius-soleus muscle was stimulated directly through several tinned pins inserted into the belly and the tendons of the muscle. Care was taken that supramaximal break shocks only were employed throughout the course of the experiment. Otherwise the technique was identical with that employed in the experiments in which nerve stimulation was used.

Results. Results were uniform in both the neuromuscular and in the direct muscle stimulation series, and are summarized in table 1. Each

intravenous injection of 5 cc. of isotonic potassium chloride was followed within a few seconds by a progressive increase in the height of the twitches, which lasted as long as injection continued and for a few seconds thereafter. The height of contractions then declined somewhat, but did not fall back to the previous level. Each successive injection thus resulted in an additional gain, until in some experiments the original height of contraction was regained and even surpassed, despite several hours of muscular twitching. In other experiments the heart stopped before the height of contraction regained its initial level. Figure 1A is taken from an experiment with neuromuscular stimulation, figure 1B from an experiment with direct muscle stimulation of a previous denervated muscle. Segments of record taken initally, after fatigue, and again after different amounts of potassium are placed side by side for purposes of comparison. The rapid recovery of

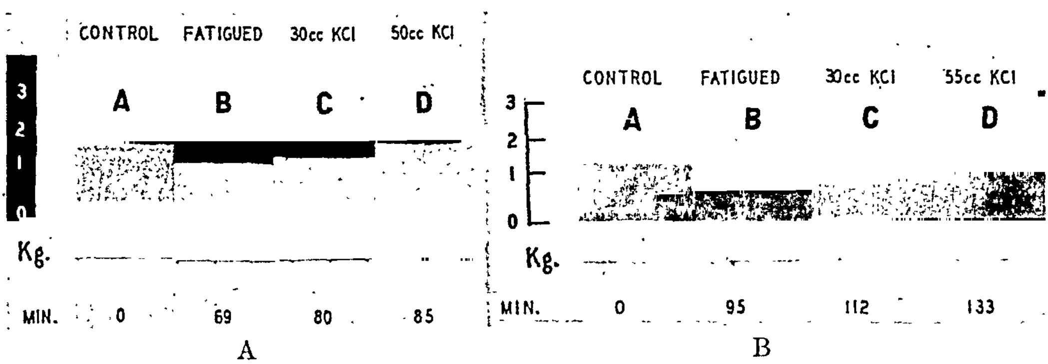

Fig. 1A. Neuromuscular stimulation. Restoration of the height of contraction of fatigued muscle following the intravenous injection of isotonic KCl. (Cat, quadriceps muscle, femoral nerve stimulated.)

Fig. 1B. Muscle (gastrocnemius-soleus) denervated 125 hours previously, stimulated directly by multiple electrodes. The intravenous injection of KCl restores the height of contraction of fatigued muscle as readily as in figure 1A.

height of twitches after potassium, in spite of further contraction and fatigue, is clearly seen in both types of experiment.

Discussion. These experiments together with those described previously (2, 3, 4, 11) indicate that an excess of potassium in the circulating fluids prevents the diminution of tension which normally occurs after a series of twitches, or, if such diminution has already occurred, restores the vigor of contraction to normal. Since it is obtainable as readily in the denervated muscles as in those stimulated through the motor nerve, this effect is evidently due chiefly to the action of potassium on the contracting muscle fibers themselves. These observations may be related to the known liberation of potassium from muscle during muscular activity of the rhythmical type studied here (5, 7, 8), which is proportional to the duration and intensity of the contraction. It has been suggested that this loss of potassium may itself be one of the factors responsible for the progressive

decrease in the intensity of contractions in fatigue (6). If this were so, anything that would favor the continuous replacement of muscle potassium might be expected to prevent muscle fatigue or to restore contractions in a muscle already fatigued. It is therefore a reasonable, though unproven, hypothesis that potassium injected intravenously restores the vigor of contraction of fatigued muscle by replacing the potassium which had been previously lost during the development of fatigue.

The observation that potassium may at times cause liberation of adrenaline (1, 10) suggests the possibility that the effect of potassium in opposing fatigue may be mediated by adrenaline. This suggestion has little to support it, since potassium injected intravenously at a slow rate causes no rise in pulse or blood pressure (9), indicating that very little if any adrenaline is liberated.

SUMMARY

The decrease in twitch tension which normally appears after a series of rhythmic twitches is prevented by the slow intravenous injection of isotonic potassium chloride. After the decrease in twitch tension has already been established the tension may be restored to normal by the same means.

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GASEOUS NITROGEN AND HELIUM ELIMINATION FROM THE BODY DURING REST AND EXERCISE

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Data are presented showing 1, the helium content of the body following a three and one-half hour exposure in a helium-oxygen atmosphere; 2, the curve of helium elimination when air or oxygen is breathed; 3, the increased rate of nitrogen and of helium elimination during exercise, and 4, the curve for nitrogen elimination when oxygen is breathed for a period of 15 hours.

A gas dissolved in the tissues of the body will diffuse into the pulmonary air spaces by way of the blood stream in proportion to the difference in partial pressure between the concentration of gas in the body and in the lungs. The breathing either of oxygen or of an oxygen-helium mixture, for example, will bring about an almost complete elimination of the dissolved nitrogen in the body. In all of our tests this principle was utilized in making gas measurements.

Eleven men in good physical condition, usually deep sea divers, served as subjects.

The helium elimination curve. The subjects breathed a gas mixture consisting of 73 to 76 per cent helium, 5 to 7 per cent nitrogen and 19 to 20 per cent oxygen for a period of three and one-half hours. During this period nearly complete saturation was attained as shown by the quantity of gas subsequently eliminated from the body following exposures of six hours' duration.

Following this saturation period the residual helium in the lungs was removed by breathing oxygen or air for a period of three minutes (lung rinsing period). The helium eliminated from the body was then measured over half-hour periods by having the subjects rebreathe either oxygen or air in a closed spirometer system of about 10 liters' capacity.

Analyses of gas samples for helium content were made in the Cady apparatus, operating on the physical principle that nitrogen and gases other than helium will be adsorbed on activated charcoal cooled to the tempera-

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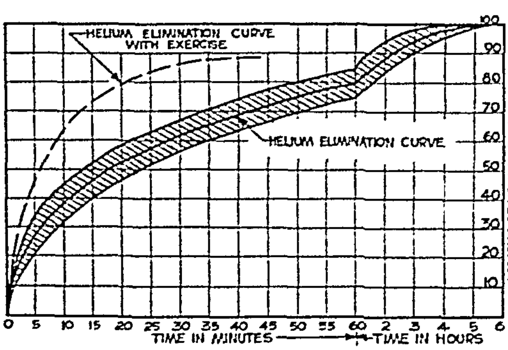
ture of liquid air. By means of a high vacuum applied to the charcoal the helium can be extracted and subsequently measured in a burette. Analyses of large samples (500 to 1000 cc.) permitted the determination of quantities of helium as small as 1.5 cc. eliminated over a period of a half-hour. With the elimination of less than 1.5 cc. in a half-hour period the end-point was considered to be reached.

The principal sources of variation and error in these experiments were: 1, the total volume of the spirometer system included a fixed value of 1600 cc. for the residual volume and dead space in the lungs; 2, the quantity of helium eliminated during the first three minutes was based in part on a measured value obtained during the third minute and on a computation involving cardiac output and helium solubility in blood. For a man



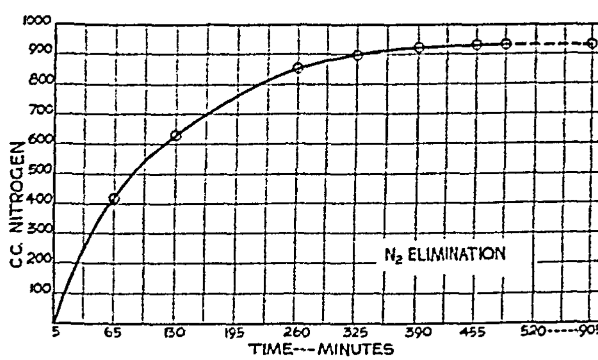


Fig. 1 (left). Percentage of helium elimination from the body plotted against time. Data obtained on 11 deep sea divers who breathed helium-oxygen mixtures for a period of 3.5 hours. Band indicates range of variation of individual results. Helium content of the body is 8.0, 1.3 cc. per kgm., 760 mm. pressure.

Broken line curve illustrates the effect of exercise on helium output.

Fig. 2 (right). Curve showing nitrogen elimination from the body of a diver, age 32, weight 154 pounds, who breathed 99 per cent oxygen in a closed system for a period of 15 hours. The nitrogen eliminated during the first 5 minutes (lung rinsing period) is not included; see table 1.

weighing 150 pounds the value computed for the helium eliminated during the first three minutes (lung rinsing period) was 75 cc. and deviation from this value was considered to be proportional to body weight; 3, an undetermined fraction of the total helium present in the body at the beginning of the saturation period was not measured by our method since it was eliminated through the skin.

The values for total helium content in duplicate tests varied less than ten per cent, and both the values for helium elimination time and helium content of the body per atmosphere of pressure were not altered appreciably by longer saturation exposures at pressures in excess of one atmosphere.

In figure 1 the helium elimination band includes the range of values

around the mean showing the minimum and maximum percentages of helium given off by men at rest in the sitting position.

Since 60 ± 5 per cent of the helium in the body leaves the tissues during the first hour, the time units are expressed in minutes up to 60. The curve is then broken and the time units for subsequent helium removal are expressed in hours.

Considerable variation in desaturation time occurred between subjects, undoubtedly due in part to the difficulty of measuring small amounts of gas collected from the body after 3 hours. Two of the thinnest men weighing 133 and 148 pounds respectively, desaturated in 4 hours in contrast with the desaturation time of 6 hours for two men weighing 202 and 206 pounds respectively.

The helium content of the body. Since it was not feasible to measure the helium tension in alveolar air during the saturation period, we computed the value from the formula,

$$\text{Tension He} = \frac{\text{He content urine}}{\text{He content equil. urine}} \times \text{B-W},$$

where B represents the barometric pressure and W the tension of water vapor.

For it has been shown by Behnke and Yarbrough (1938) that following a change in alveolar nitrogen pressure (and also in alveolar helium pressure) the urinary nitrogen pressure approaches equilibrium with the gas pressure in the lungs after a period of 30 to 60 minutes.

With the tissues of the body in equilibrium with a helium alveolar pressure corrected to 760 mm., the helium capacity of the body based on values obtained from 11 deep sea divers is 3.6 ± 0.6 cc. per pound or 8.0 ± 1.3 cc. per kilogram of body weight.

For comparison, the nitrogen content of a similar group of men was found to be 18 ± 2.0 cc. per kilogram, 760 mm. pressure (Behnke, 1937).

Effect of exercise on helium elimination. The subjects breathed a helium-oxygen mixture while exercising on a stationary bicycle for periods of 15 and 30 minutes respectively. The helium taken up by the body was then measured as in previous tests and compared with the absorption rate of control tests.

Oxygen consumption during exercise was about 3 times the quantity used in control runs, or about 4 times greater than the basal metabolic rate.

The broken line curve of figure 1 represents the values obtained. Exercise over a period of 15 minutes increased helium elimination 60 per cent compared with resting levels, and over a period of 30 minutes, 40 per cent.

In these tests the helium eliminated during the first 3 minutes was not measured, and the values were subject to considerable variation. The

results indicate that the maximum benefit from exercise is derived during the first 15 minutes. A forty-five minute period of exercise, for example, increased helium elimination only 12 per cent compared with control rest periods indicating that exercise of more than 30 minutes' duration does little to increase gas elimination from the body.

The nitrogen elimination curve. The manner of nitrogen removal from the body is shown graphically in figure 2. The data were obtained on a deep sea diver who breathed 99 per cent oxygen for a period of 15 hours while wearing a rubber helmet placed in the circuit of a double spirometer system (figure 3).

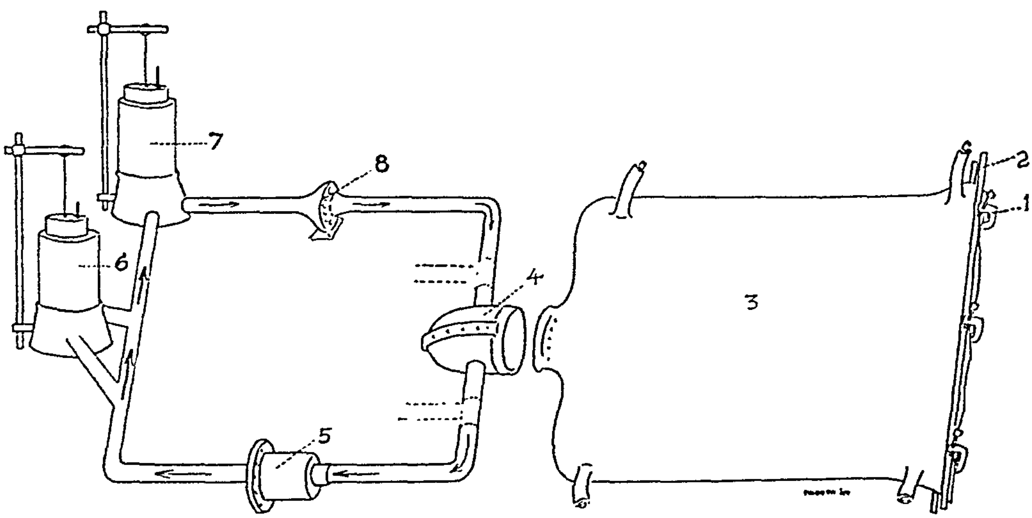


Fig. 3. Diagram of apparatus used to measure gas eliminated from the lungs and gas diffusing through the skin.

1, clamp on iron bars; *2*, used to seal open end of rubber bag; *3*, designed to fit around the body serving as a gas-tight seal; *4*, rubber helmet and collar secured with adhesive tape around the subject's neck; *5*, canister with absorbent for water and CO_2 cooled with ice; *6*, spirometer for measuring added oxygen; 7, spirometer; 8, fan to circulate oxygen, bearing encased in oil to exclude air. Dotted lines indicate connections to a second similar system.

The determination of the quantity of nitrogen eliminated from the body tissues particularly as the end-point is reached, although simple in principle, is a tedious procedure requiring principally an air-tight system for recirculating oxygen and a facepiece that can be worn comfortably for long periods of time.

Since the time limit for wearing a mask pressed tightly against the face is about 6 hours, one of the authors (A. R. B.) devised a close-fitting rubber helmet (dead space 500 cc.) secured with adhesive tape around the neck, which could be worn for a period of 17 hours without discomfort.

The additional spirometer (fig. 3) serves to measure oxygen as well as to facilitate the recirculation of gas.

At the end of each hour following a lung rinsing period of 5 minutes with tank oxygen, a shift was made to an alternate but similar double spirometer system. In this manner it was possible to detect errors incident to faulty apparatus and to maintain percentages of oxygen above 99 in the system after the first 4 hours.

Diffusion of atmospheric nitrogen into the closed circuit amounted to between 20 and 30 cc. per hour in control tests without a subject.

During a test run with a subject breathing in the helmet and surrounded by air, cutaneous absorption of atmospheric nitrogen amounted to between 15 and 25 cc. per hour. This source of nitrogen was obviated by placing the subject in a large rubber bag filled with oxygen.

With reference to the time required for nitrogen elimination, the results are in accord with data obtained by Behnke (1937) in tests conducted at the Harvard School of Public Health. It is observed that the nitrogen is eliminated rapidly during the first 2 hours and then slowly during successive hours. During a period between 9 and 12 hours an end-point within the limits of experimental error (usually ± 2.5 cc. per hr.) is reached. The results of a single test plotted in figure 1 (first 5 min. of gas elimination excepted) and representative of the data obtained on several men, are listed in table 1.

Nitrogen elimination from the body during rest and exercise periods. Control test. Following a period of three minutes of timed respiration (six deep inhalations per minute) for the purposes of eliminating the residual nitrogen in the lungs, a subject 35 years old, weighing 192 pounds, breathed oxygen through a face mask in a closed spirometer system for a period of 27 minutes daily. There was no preliminary rest period or attempt to regulate the activities of the individual. The results of six consecutive daily tests of nitrogen eliminated between 3 and 30 minutes (subject seated) are the following: 364, 332, 339, 344, 328 and 344 cc. respectively. The oxygen consumption in cubic centimeters per minute for the respective test runs was: 356, 327, 357, 352, 343 and 352.

The results indicate that in 5 out of 6 experiments sufficiently close agreement is attained so as to render the procedure satisfactory. The amount of nitrogen given off by the body during the first 3 minutes of oxygen breathing (lung rinsing period) is indeterminate and presents undoubtedly the greatest source for variation in the values.

Rest and exercise runs. In table 2 are listed the results of nitrogen elimination and of oxygen consumption during rest and exercise for periods of 3 to 13 and 3 to 30 minutes. During the rest period the subject was seated on a stationary bicycle; during the exercise period the bicycle was pedalled at a rate sufficient to increase oxygen consumption two and one half times.

In summary, the results indicate that exercise produces a hundred per

cent increase in nitrogen elimination for the period between 3 and 13 minutes and a 39 per cent increase in the period from 3 to 30 minutes.

Compared with helium test runs, exercise during the first 15 minutes results in a greater percentage elimination of nitrogen. This finding may be explained on the basis of a threefold greater fat-water solubility ratio

TABLE 1

Nitrogen elimination from a deep sea diver, age 32, weight 154 pounds, height 69 inches

(See fig. 2)

TIME	TOTAL ELIM.	N_2 DIFFUSION† INTO SYSTEM	NET N_2
min.	*cc.*		
0–5*	(150)		(150)*
5–65	481	47	434
65–70‡	(25)		(25)
70–130	228	47	181
130–135‡	(12)		(12)
135–195	158	47	111
195–200‡	(7)		(7)
200–260	122	47	75
260–265‡	(5)		(5)
265–325	85	47	38
325–330‡	(2)		(2)
330–390	72	47	25
390–395‡			(–)
395–455	53	47	6
460–520	52	47	5
525–585	47	47	
Body surrounded by oxygen			Total....1076
595–655	46		
660–720	33		
725–785	34		
790–850	24		
Body surrounded by air			
855–905	48		

* Lung rinsing period. Estimated value of nitrogen elimination for this period—150 cc.

† Diffusion of nitrogen into system includes atmospheric nitrogen diffusing through rubber tubing, helmet, spirometer water seal, and nitrogen diffusing through the skin.

‡ Represents a period of lung rinsing whenever a shift was made in spirometer systems. Nitrogen elimination values estimated for these periods.

for nitrogen compared with helium, and consequently the circulating blood has a greater nitrogen reservoir in lipid material to draw upon in comparison with available helium.

Nitrogen elimination when a helium-oxygen mixture is breathed. The addition of helium to the inhaled oxygen did not interfere with the removal

of nitrogen from the tissues of the body. In a typical test a helium-oxygen mixture was breathed for 30 minutes followed by a 90-minute period of oxygen inhalation. The nitrogen given up by the body in the period following helium inhalation was 309 cc. compared with a value of 301 cc. when oxygen was breathed during the initial 30-minute period.

When a helium-oxygen mixture was breathed for a period of 5 hours, 189 cc. of nitrogen were subsequently eliminated compared with 206 cc. eliminated when oxygen was inhaled for the corresponding period of time. Whether or not complete nitrogen removal from the body could be brought about through inhalation of combined helium and oxygen was not tested.

Application of experimental data. The elimination curves for helium and for nitrogen indicate that following saturation a helium dive will require about one-half the period of decompression necessary for an air

TABLE 2

Gaseous nitrogen elimination and oxygen consumption during periods of rest and exercise

SUBJECT	AGE	HEIGHT	WEIGHT	B.M.R.	TOTAL NITROGEN ELIMINATION				OXYGEN CONSUMPTION, CC./MIN.			
					Rest		Exercise		Rest		Exercise	
					3-13′	3-30′	3-13′	3-30′	3-13′	3-30′	3-13′	3-30′
A	35	73	182	276	145	314		417	358	420		1053
B	32	71	175	274		262		361	342	312	614	531
C	31	72	187	283	103	287	194	443	380	403	772	1049
D	29	72	168	270	119	304	268	389	349	346	693	808
E	31	71	190	277	135	271	274	534	354	328	729	649
F	31	69	163	248	157	293	270	395	328	324	973	1088
G	38	71	180	276		364		403	406	383	819	1001
H	30	74	201	297		380		498		355	973	1135

dive. For short exposures, however, we have found that the decompression time is about the same for both the air and the helium dive.

About 75 per cent of the total body nitrogen is eliminated at a comparatively rapid rate and hence does not usually contribute to the formation of "bends." There is however a relatively small amount of gas dissolved in the bone marrow that requires many hours for proper elimination. At a depth of 90 feet, for example, 10.5 hours of air decompression were required following a 9-hour exposure (probable saturation). On the other hand a 2-hour exposure (75 per cent saturation) at the same depth required only 60 minutes for decompression.

In tissues other than the bone marrow and the spinal cord, gaseous diffusion and a greater circulation of blood tend to equalize nitrogen pressure throughout the body (Behnke, Thomson and Shaw, 1935). In the bone marrow and spinal cord the greater nitrogen uptake due to high fat

content, the limitation of diffusion by bony walls, and the sluggish circulation (Campbell and Hill, 1933) appear to be the factors responsible for the slow decompression necessary after long exposures in atmospheres of compressed air. Helium possessing lesser solubility in fat compared with nitrogen should materially hasten decompression if breathed during prolonged exposures in high pressure atmospheres.

In order to augment the removal of absorbed nitrogen during the decompression of deep sea divers, either oxygen or helium-oxygen mixtures can be used. The advantage of the helium-oxygen mixture is that it is not toxic up to depths of 500 feet, in contrast with oxygen which induces toxic symptoms when inhaled at greater depths than 60 feet after prolonged exposures. On the other hand, the absorbed helium in replacing some of the nitrogen will tend to limit the speed of the diver's ascent.

The inhalation of 99 per cent oxygen supplied from commercial tanks for periods as long as 17 hours without eliciting toxic symptoms is considerably in excess of previously reported results. However, the subjects were in a state of complete relaxation, the inhaled oxygen was cooled to between 75° and 80° dry bulb temperature, and the relative humidity was maintained at about 50 per cent. It may be significant also that periods of forced respiration which were associated with substernal distress during oxygen inhalation in 6- to 8-hour tests, were suspended during the 15-hour runs.

SUMMARY

1. At a given pressure the tissues of the body will absorb about 40 per cent as much gaseous helium as nitrogen.

2. The time required for the elimination of the absorbed helium is about 50 per cent of the time required for nitrogen elimination.

3. Exercise hastens gas elimination from tissues but the value of exercise is chiefly during the first 30 minutes.

4. Gas elimination is comparatively rapid from the fluid constituents of tissues; by contrast the bone marrow containing a high percentage of fat requires from 9 to 12 hours for "decompression" after saturation with nitrogen at high pressure atmospheres.

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CUTANEOUS DIFFUSION OF HELIUM IN RELATION TO PERIPHERAL BLOOD FLOW AND THE ABSORPTION OF ATMOSPHERIC NITROGEN THROUGH THE SKIN

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It was demonstrated by Gerlach (1851) that some elimination of carbon dioxide and adsorption of oxygen was effected through the skin. Subsequent investigations by Schierbeck (1893) and von Willebrand (1902) established the concept of a critical temperature of 33°C. above which carbon dioxide output is proportional to the rise in temperature. Barratt (1897) found that carbon dioxide elimination was doubled at 35°C. compared with the output at 25°C. Shaw, Messer and Weiss (1929) demonstrated a threefold output of carbon dioxide for the same temperature difference. In a subsequent paper Shaw and Messer (1930) reported that there was a critical "effective temperature"[1] of 34° above which the rate of carbon dioxide excretion is accelerated sixfold per unit rise in "effective temperature."

Since carbon dioxide and oxygen are the essential gases in tissue respiration, it was thought worthwhile to measure the cutaneous diffusion of the inert gas, helium, in relation to temperature change and to report for the first time some values for diffusion of atmospheric nitrogen through intact skin.

Essentially we have found that there is also a critical temperature for the increased diffusion of helium through skin, and that the amount of helium absorption is directly related to peripheral blood flow.

Method of procedure. The unclothed subject lying on a cot was placed in a rubber bag with the head protruding through an air-tight collar. The bag was then inflated with tank helium after an adequate rinsing period of ten minutes.

The helium diffusing through the skin was measured as the amount of gas eliminated from the lungs during rebreathing in the helmet-double

[1] "Effective temperature" as defined by the American Society of Heating and Ventilating Engineers.

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spirometer systems described in the preceding paper. Gas samples were taken at half-hour intervals when a shift was made to the alternate helium-free spirometer system. The helium content in the bag around the body varied between 90 and 95 per cent and the helium content in the rebreathed gas was usually less than 1 per cent.

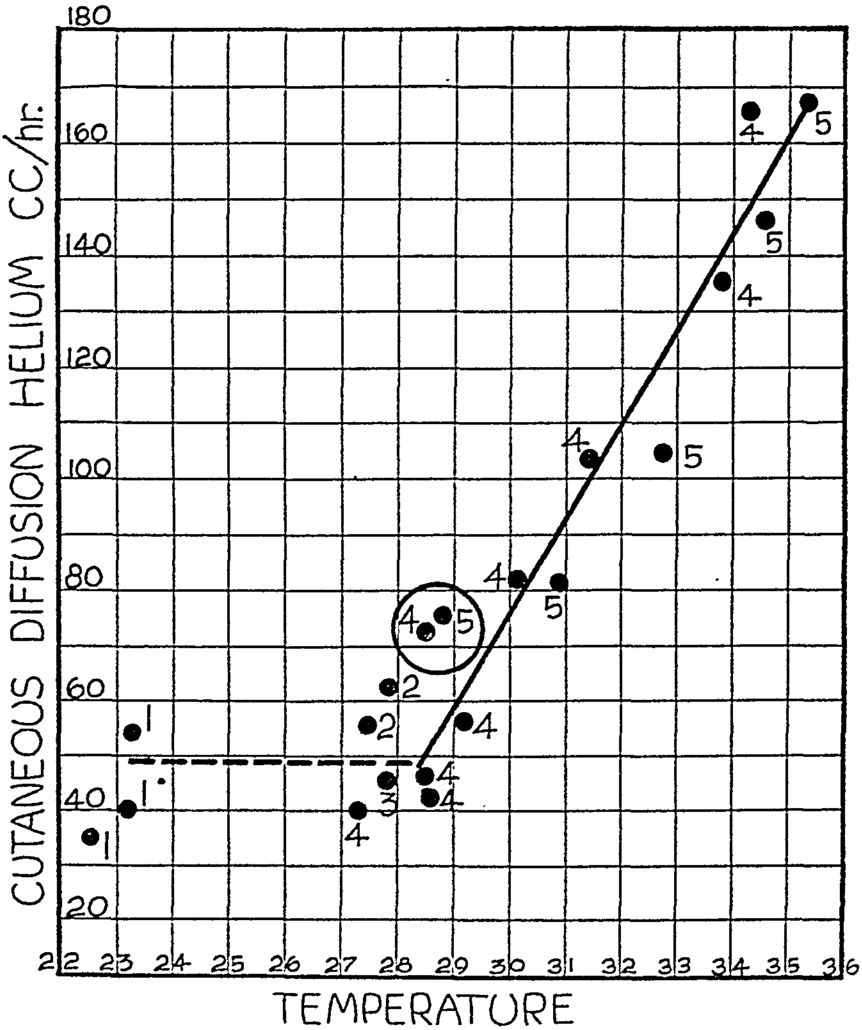

Fig. 1. Cutaneous diffusion of helium in relation to temperature, measured as cubic centimeters of helium recovered from the lungs per hour when the body is immersed in a helium atmosphere, pressure 700 mm. The numbers, 1 to 5, refer to different subjects. The encircled values were obtained after the previously heated ambient helium had been cooled to 29°C.

In the demonstration of nitrogen diffusion through skin it was first necessary to bring about nitrogen desaturation of the body in the manner described in the preceding paper. After the quantity of nitrogen eliminated from the lungs had reached a constant value for a period of several hours, the subject's body enclosed in a rubber bag was surrounded

with air or oxygen. The corresponding fluctuations in the quantities of nitrogen recovered from the lungs relative to the enveloping atmosphere indicated the amount of gaseous diffusion through the integument.

EXPERIMENTAL DATA. When the body was surrounded with helium a period of from 30 to 60 minutes was required to bring about equilibrium between the helium diffusing inward through the skin and the helium eliminated from the lungs.

In a typical test run values for successive half-hour periods following the initial bag rinsing period of 10 minutes were: 9.5, 17.5. 17.1, 20.0, 19.8, 30.7 and 23.7; when the bag helium was replaced by air or oxygen the values for the succeeding half-hour periods in this test were: 15.1, 5.6, 6.0 and 5.0.

TABLE 1

Cutaneous diffusion of helium in relation to temperature, measured as cubic centimeters of helium eliminated from the lungs per hour when the body, enclosed in a rubber bag, is surrounded by helium (700 mm. pressure)

Continuous tests on two subjects

	RUBBER BAG HEATED				BAG COOLED
	Time in minutes from start of helium exposure				
	40–100	100–160	160–220	220–280	365–425
Subject 4, age 31, ht. 65.5 in., weight 140 lb.	S.A. 1.7 sq. m.				
Temperature of bag gas	30.2	31.5	33.9	34.2	28.8
Cubic centimeters He from lungs	82	104	138	167	74
Subject 5					
Temperature of bag gas	31	33	34.5	35.8	29
Cubic centimeters He from lungs	81	105	146	173	75

When the temperature of helium in the bag was 27°C. to 28°C. about 40 to 60 cc. of helium diffused through the skin per hour (fig. 1), helium pressure in the bag being approximately 700 mm.

When the helium in the bag was heated to 35°C. cutaneous diffusion of helium increased to 170 cc. per hour and returned to a value of 70 to 75 cc. per hour when the ambient helium atmosphere was cooled to 29°C. In table 1 the results of continuous tests on two subjects are recorded. The temperature values may be regarded as "effective temperatures" since accumulated moisture in the bag and the profuse sweating indicated a saturated atmosphere.

DISCUSSION. In these tests we are satisfied to show that helium diffuses inward through the skin and that there is a linear increase in the diffusion of helium as measured by the amount of gas eliminated from the lungs,

beginning at 28 to 29°C. and amounting to a two and one-half-fold increase at 36°C. compared with the initial value.

Between 22°C. and 28°C. the diffusion rate follows a plateau but the data are insufficient to establish a quantitative relationship. Apart from an increase in peripheral blood flow, temperature rise augments gaseous diffusion through tissue at the rate of one per cent per degree rise starting with 20°C. as unity (Krogh, 1919); this thermal effect however is within the limits of our experimental error.

We do not consider that a close correlation exists between any particular temperature value in table 1 and the corresponding value for helium absorption since there is a time interval required before equilibrium is established between cutaneous diffusion and the elimination of the absorbed gas from the lungs.

Helium diffusion in relation to peripheral blood flow. The abrupt linear augmentation of helium diffusion in the range of 28°C. and upward is explained chiefly on the basis of increased cutaneous blood flow. Having worked out a simple relationship between heat loss and peripheral blood flow, Hardy and Soderstrom (1938) estimated that for the nude, motionless body, blood flow to the skin increases about threefold between 28°C. and 35°C. Below a temperature of 28°C. blood flow was minimal and constant.

Of prime interest is the close correspondence between the computation of peripheral blood flow on the basis of heat loss from the body and on the basis of helium diffusion through the skin. Under the conditions of their tests, Hardy and Soderstrom found that at 35°C. the blood flow to the skin was about 13 liters per hour per square meter of skin surface. In our tests performed under similar conditions with regard to the resting state, values of the same order as those computed by Hardy and Soderstrom were obtained. If, for example, about 170 cc. of helium are recovered from the lungs per hour (table 1), then about 20 liters of blood would be required to transport this quantity of helium from the periphery to the lungs. This calculation is based upon the following considerations, that under equilibrium conditions 1 liter of blood will hold in solution about 8 cc. of helium at a pressure of 700 mm. (Hawkins and Shilling, 1936). Dividing the hourly value of helium eliminated from the lungs by the solubility value for helium in blood gives a quantity indicative of peripheral blood flow.

The surface area of subject 4 (table 1) according to the DuBois height-weight chart is 1.7 sq. m. Excluding the subject's head and that part of the body in contact with rubber and perhaps not entirely available for gaseous absorption, it is probable that the effective area through which the 20 liters of blood flowed was at least 1.2 sq. m. It would appear that measurements of helium diffusion carefully correlated with skin and ambient gas temperatures should give accurate values for peripheral blood flow.

It may be of interest to record that diffusion of helium outward through the skin when a helium-oxygen mixture is inhaled, is of the same order as inward diffusion of helium. Our tests are too few in number however to form a comparison.

Cutaneous nitrogen diffusion. The demonstration that nitrogen diffuses inward through the skin is made possible only by rendering the tissues of the body relatively nitrogen-free as described in the preceding paper and then by surrounding the body either with air or oxygen. In table 2, column 1, for example, the value of 53 represents the quantity of nitrogen in cubic centimeters accumulating in the closed system for oxygen inhala-

TABLE 2

Diffusion of nitrogen through skin as indicated by the hourly values for nitrogen elimination from the lungs when the body is surrounded by air or oxygen

TIME FROM BEGINNING OF TEST	CC. NITROGEN RECOVERED FROM LUNGS PER HOUR		
	(1) Subject 1*	(2) Subject 2*	(3) Subject 2†
hrs.			
7.5	53 Body in air		
8.5	52		
9.5	47	55 Body in O_2	73 Body in air
11.0	46 Body in O_2	49	62
12.0	33	47	49 Body in O_2
13.0	34	46	
14.0	24	41	68 (2 hrs.)
15.0	48 Body in air	60 Body in air	37
16.0		67‡	
17.0			64 (2 hrs.)

* Subjects breathed 99 per cent oxygen throughout test period.

† Subject breathed helium-oxygen mixture first 4 hours, then oxygen for remainder of test period.

‡ Air around the body heated during last half-hour.

tion during the period between 6.5 and 7.5 hours. This value includes atmospheric nitrogen diffusing into the rubber helmet, tubing, and spirometer water seals, nitrogen diffusing through the skin, and residual nitrogen from the tissues of the body including perhaps a small amount of gas absorbed by the blood stream from the alimentary tract.

Control runs without a subject indicated that between 20 to 30 cc. of nitrogen were absorbed from the atmosphere into the oxygen system per hour. When the body was surrounded by oxygen, nitrogen elimination (column 1) decreased from 47 cc. to a quantity between 24 cc. and 34 cc. only to return to a value of 48 cc. when the body was again surrounded by air.

The data in table 2 indicate, therefore, that between 15 and 25 cc. of atmospheric nitrogen (pressure approximately 600 mm.) diffuse inward per hour through a total skin area of 1.72 sq. m. of which about 0.56 sq. m. of surface was in contact with the rubber bag.

Under similar conditions in comparison with the rate of helium diffusion, nitrogen uptake is somewhat less than 50 per cent. This decreased *cutaneous absorption rate for nitrogen prevents the attainment of equilibrium* between the nitrogen pressure in cutaneous vessels and in the ambient atmosphere.

SUMMARY

1. Diffusion of helium inward through the skin shows a linear increase with temperature in the range of 28°C. to 35°C.

2. This increase in helium absorption can be correlated with the rise in peripheral blood flow above 28°C.

3. Computation of peripheral blood flow on the basis of helium transport from the periphery to the lungs gives values of the same order as those computed by Hardy and Soderstrom on the basis of heat loss from the body.

4. The diffusion of atmospheric nitrogen inward through the skin has been demonstrated for the first time and is somewhat less than 50 per cent of the amount of helium absorbed under similar conditions.

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NITROGEN ELIMINATION AND OXYGEN ABSORPTION AT HIGH BAROMETRIC PRESSURES

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Measurements of gaseous nitrogen given up by the tissues of the body when oxygen is inhaled at pressures above one atmosphere have not been previously made with the exception of some tests carried out by Campbell and Hill (1931). The purpose of the experiments reported in this paper was to determine the pressure level at which maximum elimination of nitrogen from the body takes place after previous exposure to a high barometric pressure.

METHOD OF PROCEDURE. Following a rest period of 30 minutes deep sea divers continuing at rest were exposed in a steel chamber to a pressure of 44.5 pounds gauge (4 atms. absolute) equivalent to a sea water depth of 100 feet for periods of 60 and 75 minutes. On the basis of previous measurements it was estimated that under these conditions and excluding the gas absorbed during the first 5 minutes, 50 and 55 per cent saturation of body tissues with excess atmospheric nitrogen was effected. It was assumed that under these conditions the degree of saturation of a given individual was comparatively uniform from day to day.

The rebreathing apparatus (fig. 1) for measuring nitrogen elimination and oxygen consumption under increased barometric pressure consisted of a two-opening rubber bag of 110-liter capacity connected by rubber tubing one inch in diameter to a canister containing a carbon dioxide and moisture absorbent. A spirometer of the Benedict type was incorporated in the system for measuring and recirculating the gas. A face mask and valves completed the circuit.

In a typical experiment 50 liters of oxygen were measured into the system which had been rinsed previously to ensure a concentration of 99 per cent. The subject having been exposed to an excess air pressure of 44.5 pounds per square inch for a period of 75 minutes, breathed oxygen for 3 minutes at the rate of 6 maximum respiration cycles per minute for the purpose of removing lung residual air. The subject was then transferred to the closed-circuit system and the barometric pressure was lowered at the rate of 25 or 50 feet per minute to the level designated for the measurement of nitrogen output.

At the end of the 27-minute period of oxygen inhalation it was found safe to reduce the pressure to one atmosphere. The subject was then transferred to a second closed circuit system for additional measurements of nitrogen output and oxygen consumption.

Periodic withdrawal of gas samples permitted the calculation of eliminated nitrogen. The volume change in a system indicated the quantity of oxygen absorbed, a correction being made for the nitrogen diffusing into the system from the body.

Experimental data. In table 1 the data refer to 8 test runs on one subject exposed to an air pressure of 44.5 pounds gauge (100 feet) for 75 minutes. It is observed that during the first 27-minute period of oxygen inhalation, there is a maximum elimination of nitrogen between the 50 and

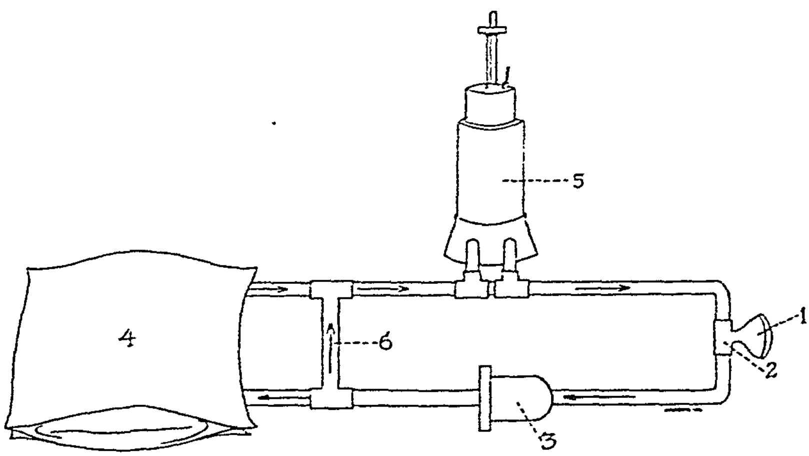

Fig. 1. Diagram of a closed system devised for the inhalation of oxygen to permit measurements of nitrogen elimination at high barometric pressures. *1*, face mask; *2*, valves; *3*, canister containing CO_2 and water absorbent; *4*, 110-liter rubber bag; *5*, spirometer for measurement of gas volume and oxygen consumption.

60-foot levels and a decreased elimination of nitrogen above and below these levels. Of the nitrogen eliminated during the first 27 minutes, approximately six-tenths of the total is given off during the first 10 minutes (3′–13′); of the total amount given off in 84 minutes approximately six-tenths is eliminated during the initial 27-minute period.

Particular significance is attached to the final experiment, table 1. It is observed that nitrogen output at the 100-foot level is 24 per cent lower than at the 60-foot level in a preceding test run; subsequently at the 50-foot level nitrogen output in this test was 73 per cent greater than at the surface level in preceding tests.

In table 2 the values were obtained on subjects who reclined on a mattress in a state of complete rest in contrast with the values obtained on subject C (table 1) who sat in a chair. It is believed that altered posture

TABLE 1

Nitrogen elimination and oxygen consumption of one subject shown at levels from 100 to 30 feet and at surface, following uniform exposure of seventy-five minutes at 100 feet pressure

Subject: C.

DATE	EXPOSURE	STOP	NITROGEN ELIMINATED (CC.)						OXYGEN CONSUMED (CC. PER MIN.)		
			At stop		At surface			Total 3-90′	3-30′	33-60′	60-90′
			3-13′	3-30′	33-60′	60-90′	33-90′				
		ft.									
5/19/39	75 min.: 100 ft.	30	798	1322	728	330	1058	2380	337	262	390
4/24/39	75 min.: 100 ft.	40	831	1335					386		
4/19/39	75 min.: 100 ft.	50	861	1527	544	430	974	2501	377	371	359
5/11/39	75 min.: 100 ft.	55	1023	1534	430	297	727	2261	296	364	361
4/20/39	75 min.: 100 ft.	60	952	1583	560	354	914	2497	369	365	373
5/ 8/39	75 min.: 100 ft.	80	817	1463					405		
5/ 9/39	75 min.: 100 ft.	90	919	1364					455		
5/10/39	75 min.: 100 ft.	100	689	1196	967*	374	1341	2537	558	328	281

* Elimination at 50 ft. level.

TABLE 2

Nitrogen elimination and oxygen consumption of four subjects shown at levels of 100, 50, 20 feet and at surface, following uniform exposure of seventy-five minutes at 100 feet pressure

Mean values given in event of two or more identical tests

SUBJECT	EXPOSURE	STOP	TESTS	NITROGEN ELIMINATED (CC.)			OXYGEN CONSUMED (CC. PER MIN.)	
				At stop 3-30′	Surface 33-90′	Total 3-90′	3-30′	33-90′
		ft.						
S.	75 min.: 100 ft.	20	1	1478	834	2312	232	226
	75 min.: 100 ft.	50	2	1533	957	2590	228	321
	75 min.: 100 ft.	100	2	1415	739	2154	254	260
Z.	75 min.: 100 ft.	20	2	1127	777	1904	214	216
	75 min.: 100 ft.	50	3	1220	809	2029	191	257
	75 min.: 100 ft.	100	1	849	785	1634	361	207
W.	75 min.: 100 ft.	20	1					
	75 min.: 100 ft.	50	1	1587	982	2569	272	300
	75 min.: 100 ft.	100	1	1486	949	2435	316	266
D.	75 min.: 100 ft.	20	1	1081	687	1768	219	264
	75 min.: 100 ft.	50	1	1079	674	1753	208	281
	75 min.: 100 ft.	100	2	1010	754	1764	255	256

may have influenced the results. In addition, pressure was lowered at the rate of 25 feet per minute to the level designated for nitrogen elimination in contrast with a rate of 50 feet per minute (table 1).

Some of the values in table 2 again indicate that a 50-foot level is optimum for nitrogen elimination, while other data show no significant difference between the several levels.

With reference to oxygen consumption the values are fairly constant for depths up to 70 feet. At levels deeper than 70 feet oxygen consumption is apparently increased occasionally as much as 58 per cent, followed by an apparent decrease in oxygen consumption as high as 20 per cent during the succeeding hour at the surface level.

TABLE 3

Nitrogen elimination and oxygen consumption of one subject shown at surface, 44, 50 and 66 feet with subsequent surface measurements, following uniform exposure of thirty minutes at 100 feet pressure

Subject: S.

DATE	EXPOSURE	STOP	NITROGEN ELIMINATED (CC.)			OXYGEN CONSUMED (CC. PER MIN.)	
			At stop 3–30′	Surface 33–90′	Total 3–90′	3–30′	33–90′
		ft.					
3/22/39	30 min.: 100 ft.	0	626	401	1027	274	325
3/24/39	30 min.: 100 ft.	0	1191	499	1690	284	343
3/27/39	30 min.: 100 ft.	0	892	856	1748	269	345
3/28/39	30 min.: 100 ft.	0	1147	511	1658	270	337
3/31/39	30 min.: 100 ft.	44	1343	548	1891	280	309
4/ 3/39	30 min.: 100 ft.	50	1312	565	1877	276	308
3/30/39	30 min.: 100 ft.	66	1341	522	1863	290	327

In a third series of tests one subject was exposed for periods of 30 minutes at a simulated depth of 100 feet. Following a 3-minute lung rinsing period and with the subject breathing in the closed circuit, decompression was effected to the surface or to intermediate levels at the rate of 50 feet per minute.

It should be borne in mind in the interpretation of results (table 3) that periods of exposure of 30 minutes or longer at a depth of 100 feet followed by a 2-minute period of decompression to the surface may give rise to "bends" (air embolism). It is possible that bubble formation in the blood stream might retard nitrogen elimination through a sudden reduction of the pressure head for nitrogen diffusion.

Indicative of bubble formation are the results obtained on March 27 when the values for nitrogen elimination in the first and second periods approached equality in contrast with the results obtained in tests where

stops were made at 44, 50 and 66 feet. In these last three tests decompression was considered to be ample by reason of the oxygen inhalation at high levels, and bubble formation may be considered as highly improbable.

In the tests featured by rapid decompression to the surface bubble formation was manifest by the onset of pruritus and the sudden, excessive fatigue occurring 3 to 5 hours later. These symptoms usually constitute early manifestations of "bends."

DISCUSSION AND APPLICATION OF EXPERIMENTAL DATA. Nitrogen elimination under favorable conditions follows an exponential type of curve with a sharp slope during the first half-hour period. Equal quantities of gas eliminated in the first and second periods bring about a flattening of the curve which suggests either bubble formation replacing a state of supersaturation in the blood stream or a slowing of the circulation induced by oxygen.

The progressive decrease in nitrogen elimination at levels between 60 and 100 feet corresponds closely to the pressure at which oxygen becomes increasingly toxic. One of the striking effects of oxygen at these levels is intense vasoconstriction manifested by facial blanching. It is highly probable that an altered or impaired blood flow consequent upon vasoconstriction is responsible for decreased nitrogen output.

Tolerance to oxygen at high pressures shows considerable variation in different individuals. In the susceptible person typical symptoms are nausea, irritability, and a sense of impending disaster. These symptoms may be followed by violent tonic and clonic muscular spasms comparable to an epileptic seizure (Behnke et al., 1935). At a pressure of 3 atmospheres (66 ft.) narrowing of the visual fields and a decrease in visual acuity are characteristic symptoms.

One of our divers developed an idiosyncrasy for oxygen following repeated exposures at a pressure of two and one-half atmospheres. In contrast to the usual vasoconstriction, an erythema of the face and neck was associated with the inhalation of oxygen even at atmospheric pressure. Subsequently this diver exhibited an allergic type of dermatitis not related to protein sensitivity. Complete remission of symptoms followed the administration of histaminase (Torantil, Winthrop).

It is this variation in susceptibility of different individuals to the toxic effect of oxygen that may account for the different values for nitrogen elimination observed at the 100-foot depth. Another factor for consideration is the well established relationship between carbon dioxide inhalation and oxygen toxicity (Shaw et al., 1934). The effect of oxygen on the elimination rate of nitrogen from individuals completely at rest (table 2) may differ from the effect on an individual in the sitting position possibly on the basis of altered carbon dioxide production.

With regard to the phenomenon of increased oxygen absorption at depths

between 70 and 100 feet (table 1), it is probable that oxygen taken up in solution by the tissues accounts for the increase. In dogs the oxyhemoglobin was not reduced in the venous blood when oxygen was breathed at a depth of 66 feet (Behnke et al., 1934) and Campbell (1929–1930) has demonstrated the great increase in oxygen pressure in the tissues of rabbits at high barometric pressures. The capacity of the tissues to absorb oxygen is about 2.3 volumes per cent for fluids and 11.3 volumes per cent for fat.

These results are of practical application in the decompression of divers. Oxygen inhalation may be started at the 100-foot level and maintained for a period of 30 minutes provided that carbon dioxide is rigidly excluded. Nitrogen elimination however may be retarded at this level because of the toxic effect of oxygen. Safe practice permits prolonged oxygen inhalation at the 50-foot level which under the conditions of our tests is associated with the maximum output of nitrogen from tissues.

During the salvage operations incident to the U. S. S. Squalus disaster, oxygen inhalation at the 50-foot level proved effective in preventing serious injury to divers exposed to high pressure atmospheres (Behnke and Willmon, 1939).

SUMMARY

1. Excess nitrogen gas absorbed by divers exposed in a compression chamber to a simulated depth of 100 feet is usually eliminated more rapidly when oxygen is breathed at a level between 50 and 60 feet in comparison with higher or lower levels.

2. The retardation in the elimination of nitrogen observed at the 100-foot depth when oxygen is breathed is attributed to the vasoconstriction and slowed circulation incident to oxygen inhalation.

3. The increased oxygen absorption accompanying oxygen inhalation at high pressures can be accounted for on the basis of oxygen taken up by the tissues in physical solution.

Acknowledgment. The authors are deeply appreciative of the outstanding technical assistance rendered by F. L. Westbrook, Chief Pharmacist's Mate, U. S. Navy.

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THE DEPENDENCE OF THE CARBOHYDRATE, FAT AND PROTEIN APPETITE OF RATS ON THE VARIOUS COMPONENTS OF THE VITAMIN B COMPLEX

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Results of previous self-selection experiments have demonstrated the dependence of the carbohydrate, fat and protein appetite of rats on the intake of three of the crystalline components of the vitamin B complex—thiamin chloride, riboflavin and nicotinic acid—and on an extract (Frost and Elvehjem) of W factor (Richter and Barelare, 1939). These experiments have now been repeated without W factor, but with a fourth vitamin B component, B_6, which since then has been crystallized (Stiller, Keresztesy, and Stevens, 1939; Kuhn, Wendt and Westphal, 1939).

It had previously been found that rats grew and thrived on a diet which they themselves selected entirely from an assortment of purified (or nearly purified) substances, offered in separate receptacles (Richter, Holt and Barelare, 1938). This assortment contained at least one representative of each of the substances known to be needed for normal nutrition:

Sucrose	Carbohydrate
Olive oil	Fat
Casein (purified and autoclaved)	Protein
Sodium chloride, 3 per cent	Sodium
Dibasic sodium phosphate, 8 per cent	Phosphorus
Potassium chloride, 1 per cent	Potassium
Calcium lactate, 2.4 per cent	Calcium
Cod liver oil	Vitamins A and D
Dried baker's yeast	Vitamin B complex
Wheat germ oil	Vitamin E

The amount of each one of these substances received by the rats depended entirely on appetite, that is, on individual selections. That the rats actually made beneficial selections we know from the fact that on such self-selection diets they grew and reproduced as well as on our standard McCollum diet, while consuming from 15 per cent to 40 per cent less food as measured in grams. Clearly, they must have eaten only needed

substances. Thus, the rats' voluntary intake or appetite provided a measure of their nutritional needs for the various food substances, minerals and vitamins. With this method studies have already been made on the nutritional needs of pregnancy and lactation (Richter and Barelare, 1938) and of experimental glandular deficiencies, produced by adrenalectomy (Richter, 1937), parathyroidectomy (Richter and Eckert, 1939), and pancreatectomy (Richter and Schmidt, in press).

In the present experiments the rats were kept on the basic self-selection diet, including the 10 substances listed above, except yeast and wheat germ oil—that is, a diet which lacked all components of the vitamin B complex—and were given access to thiamin chloride, riboflavin, nicotinic acid, and B_6 singly and in combinations. In order to obtain information regarding the effects of these different components on the endocrine glands, the rats were killed and autopsied at the end of 40 days on the self-selection diet, that is, when they had reached an average age of approximately 105 days.

METHODS. In these experiments we followed our standard technique used for metabolic and endocrinologic studies. At an average age of 45 days female rats were placed in individual cages, containing a revolving drum with a cyclometer plus a living compartment with a food cup (McCollum diet) and a graduated inverted water bottle. Daily records were taken of activity, food and water intake, and of the vaginal smears. The animals were weighed weekly. After approximately 15 days when base lines had been obtained, the rats were placed on the self-selection diet. This was simply accomplished by removing the small living compartment with the single food cup and water bottle and replacing it with a larger cage which had space for 3 food cups and 8 to 16 bottles for the solutions used in the self-selection diet. After 40 days on these self-selection diets the rats were killed and autopsied. At autopsy all of the endocrine glands were weighed and preserved for histological study.

In these experiments the basic self-selection diet offered the following substances, ad libitum, in separate containers: *solids in food cups*—casein (purified and autoclaved), sucrose; *fluids in bottles*—olive oil, sodium chloride, 3 per cent, dibasic sodium phosphate, 8 per cent, potassium chloride, 1 per cent, calcium lactate, 2.4 per cent, magnesium chloride, 0.5 per cent, cod liver oil, tap water.

One negative control group of 4 rats had access only to this basic diet, while a positive control group of 8 rats had access to dried baker's yeast, in addition to the basic diet, that is, to an assortment of substances from which we had previously found that rats made selections which resulted in normal growth.

The nine experimental groups, usually composed of 4 rats each, had

access to the substances listed above and to thiamin chloride, riboflavin, B_6, and nicotinic acid, singly and in combinations as follows:

1. Thiamin chloride
2. Riboflavin
3. B_6
4. Nicotinic acid
5. Thiamin chloride plus riboflavin
6. Thiamin chloride plus B_6
7. Riboflavin plus B_6
8. Thiamin chloride plus riboflavin plus B_6
9. Thiamin chloride plus riboflavin plus B_6 plus nicotinic acid

Stock solutions[1] of the 4 vitamins (made fresh weekly) were kept in a refrigerator. All food cups and bottles were thoroughly cleaned and refilled twice weekly.

RESULTS. *Preliminary experiments.* The preliminary experiments confirmed our previous experience and showed in addition that rats will take vitamin B_6 and that its ingestion affects protein appetite, growth, and vaginal smears. For these experiments we used 4 rats which for 100 days had been on the self-selection diet, without access to yeast, but with access to thiamin chloride, riboflavin, and nicotinic acid. At this time the rats were underweight, showed dioestrous vaginal smears, and for two months or more had eaten only small amounts of casein and only moderate amounts of sucrose, but large amounts of olive oil. When a 0.02 per cent solution of vitamin B_6 was offered, all the rats took from 1 cc. to 4 cc. per day. The rats gained weight, their vaginal smears again showed cornified cells, however, not with the normal regularity, and they ate larger amounts of protein.

Final experiments. Intake of the 4 vitamins. The vitamins were offered to the rats in the following concentrations: thiamin chloride, 0.02 per cent; riboflavin, 0.0025 per cent; nicotinic acid, 0.1 per cent; B_6, 0.02 per cent. These particular concentrations were chosen empirically on the basis of our own taste.

The rats drank freely of these vitamin solutions. They took considerably more of each than the generally recognized maintenance doses. This may have been due either to the loss of some of the solution in the process of drinking or to a slight deterioration of the solution between changes. Table 1 summarizes the results. At the left it gives the 9 different vitamin choices and in the 4 columns the average daily intake and range of variation of thiamin chloride, riboflavin, B_6, and nicotinic acid in milligrams for the 40-day period.

[1] We wish to express our gratitude to Merck and Company, Inc. for their generous supply of the vitamins used in these experiments.

Carbohydrate, fat and protein appetite. Figure 1 summarizes these results. It gives the average daily carbohydrate (sucrose), fat (olive oil), and protein (casein) intake for the last 20 days on the diets of the two control groups of rats, one offered the basic diet with yeast and one the basic diet without yeast, and also for the nine experimental groups which had access to the basic diet and to thiamin chloride, riboflavin, B_6, and nicotinic acid, singly or in various combinations. The control and experimental

TABLE 1

Average daily intake of vitamins for 40-day period in milligrams

	B_1	RIBOFLAVIN	B_6	NICOTINIC ACID
B_1	0.72 (0.39–1.02)			
Riboflavin		0.11 (0.02–0.20)		
B_6			0.30 (0.05–1.01)	
Nicotinic acid				1.02 (0.91–1.10)
R, B_6		0.08 (0.04–0.11)	0.54 (0.31–0.78)	
B_1, R	0.57 (0.40–0.87)	0.11 (0.09–0.13)		
B_1, B_6	0.30 (0.14–0.53)		0.61 (0.25–1.13)	
B_1, R, B_6	0.65 (0.13–1.67)	0.11 (0.03–0.17)	0.39 (0.33–0.46)	
B_1, R, B_6, N.A.	0.56 (0.24–1.41)	0.17 (0.11–0.26)	0.66 (0.54–0.75)	0.83 (0.49–1.02)

groups are each listed in the order of intake of each of the three foodstuffs. A comparison of the sucrose intake records of the two control groups (solid black rectangles) shows that the rats with yeast ate large amounts (6.6 grams per day), while rats with no yeast ate only minimal amounts (1.3 grams). The riboflavin rats ate only a very small amount of sucrose (0.9 gram), even less than the no-yeast group. The B_6, nicotinic acid, and riboflavin plus B_6 rats showed only slightly larger appetites for sucrose. On B_1 alone or on the combination of B_1 with the other vitamins the sucrose

appetite was always good. On all 4 vitamins the rats ate almost as much sucrose (5.5 grams) as on the full self-selection diet with yeast.

In general, the olive oil or fat appetite varied inversely with the sucrose and casein appetites. The riboflavin rats, which ate the smallest amounts of sucrose (0.8 gram) and casein (0.2 gram), ate the largest amounts of olive oil (2.3 cc.), while the group of rats on all 4 vitamins, which had next to the highest sucrose and casein appetites, had next to the smallest olive oil appetite (0.8 cc.).

The protein appetite of the 2 control groups showed the widest variation of any of the 3 substances. The full self-selection, or yeast, group had an average intake of 3.2 grams, while the no-yeast rats averaged only 0.4 gram. On riboflavin, nicotinic acid, or B_6 alone, or on B_1 and B_6, or on riboflavin and B_6, the protein appetite remained minimal. The protein appetite appears to depend much more on combinations of the vitamins

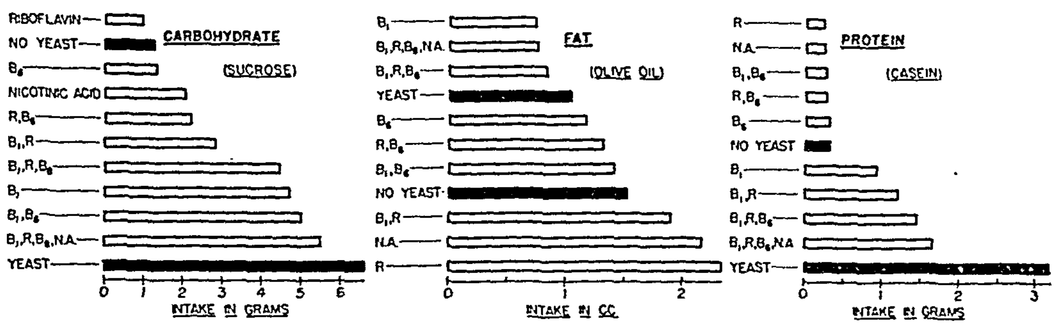

Fig. 1. Graph showing the average daily intake, for the last 20 days on diet, of carbohydrate (sucrose), fat (olive oil), protein (casein) for the 2 control (blacked rectangles) and 9 experimental groups of rats. For each of the three foodstuffs the different groups are listed in order of their average daily intake.

than on any single vitamin, unless possibly it be thiamin. Thiamin and riboflavin constitute the most important double combination, for without these two vitamins the protein appetite remained very low. B_6 added to this combination increased the protein appetite still more, and the addition of nicotinic acid increased it still further, but the combination of all 4 vitamins failed to increase the protein appetite to the average for the rats on the full self-selection diet. Clearly, the protein appetite depends on still other substances present in yeast and not offered in these experiments, such as, for instance, choline, nucleic acid or pantothenic acid.

Table 2 brings out another aspect of these results. It gives the average daily caloric intake of carbohydrate, fat and protein for the last 20 days on the self-selection diet and the average daily total calories. This table lists the various vitamin combinations in order of the average daily caloric intake. It also gives the caloric percentages of the three foodstuffs. The total caloric intake ranged from 17.0 calories for the B_6 group to 36.5

calories for the rats on all 4 vitamins, and 48.5 calories for the control or yeast group of rats. The caloric intake remained low for all the single components as well as for a double component (riboflavin plus B_6) which did not include thiamin chloride.

The second part of the table shows that carbohydrate percentages ranged from 14.3 for the riboflavin rats to 65.3 for the thiamin chloride rats, while the fat percentages varied from 18.2 for the rats on all 4 components to 82.4 for the rats on riboflavin, and the protein percentages ranged from 3.2, 3.3, 3.6 for rats on B_1 plus B_6, riboflavin, and nicotinic acid respectively to 25.8 for the control group of rats on yeast. The percentages of the rats on all 4 components most closely approximated those of the yeast group. The protein percentages showed the greatest discrepancy, 19.0 as compared to 25.8.

TABLE 2

Average daily caloric intake for last 20 days on diet

	CARBOHYDRATE	FAT	PROTEIN	TOTAL	CALORIC PERCENTAGES		
					Carbohydrate	Fat	Protein
B_6	5.8	10.0	1.2	17.0	34.1	59.1	6.8
No yeast	4.8	13.0	1.5	19.3	24.9	67.6	7.5
R, B_6	8.8	11.2	1.1	21.1	41.8	53.0	5.2
Riboflavin	3.5	19.8	0.8	24.1	14.3	82.4	3.3
Nicotinic acid	8.6	18.5	1.0	28.1	30.6	65.8	3.6
B_1	19.2	6.3	3.9	29.4	65.3	21.6	13.1
B_1, R, B_6	18.2	7.2	5.9	31.3	58.2	23.0	18.8
B_1, R	11.5	16.1	4.9	32.5	35.4	49.6	15.0
B_1, B_6	20.7	12.0	1.1	33.8	61.2	35.6	3.2
B_1, R, B_6, N.A.	22.9	6.7	6.9	36.5	62.8	18.2	19.0
Yeast	27.1	8.9	12.5	48.5	55.8	18.4	25.8

Mineral appetite. Table 3 gives the intake of mineral solutions. The intakes of calcium lactate, dibasic sodium phosphate and magnesium chloride of the rats on the various vitamin diets are of particular interest. Their intakes were low in the no-yeast control group and high in the full self-selection or yeast control group. Single vitamins did not have much effect on the appetite for these minerals, but the combination definitely increased it. The group on all 4 vitamins had the highest calcium lactate intake of any of the combinations. The other minerals—sodium chloride and potassium chloride—did not show any consistent changes. Since the rats on 3 or all 4 vitamins showed greatest growth of any of the experimental groups, the increased calcium and phosphorus appetite may be understood as responses to greater needs for these minerals used in skeletal growth.

Effects produced on body weight. Figure 2 summarizes the results produced by the various vitamins on growth. It gives the weights at the end of the 40 days as per cent of the weight at the start. On the full diet (yeast) this averaged 135 per cent; on the no-yeast diet, 62 per cent. On nicotinic acid, on riboflavin, on riboflavin plus B_6, and on B_6 alone the

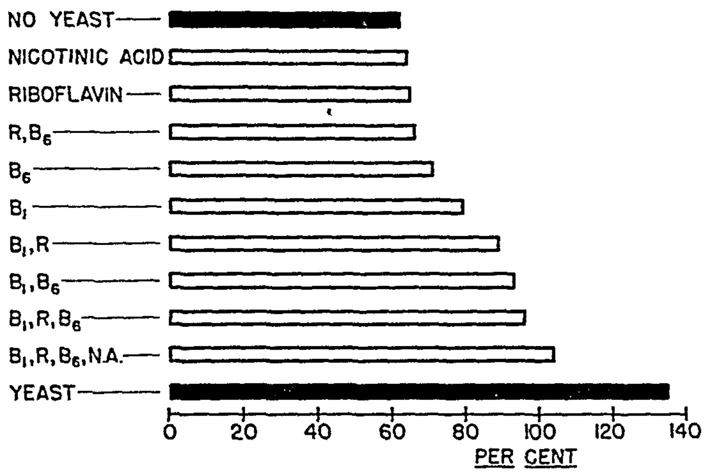

Fig. 2. Graph showing the effects produced by the various diets on body weight. It gives for the 2 control (blacked rectangles) and 9 experimental groups of rats the average body weight at the end of the 40-day period expressed as per cent of the average weight present at the start.

TABLE 3

Average daily intake of minerals for last 20 days in cubic centimeters and grams

	Calcium lactate		Sodium chloride		Sodium phosphate		Magnesium chloride		Potassium chloride	
	cc.	*gram*	*cc.*	*gram*	*cc.*	*gram*	*cc.*	*gram*	*cc.*	*gram*
No yeast	2.6	0.06	2.0	0.06	1.4	0.11	1.5	0.008	3.2	0.03
B_1	1.6	0.04	3.1	0.09	1.4	0.11	1.7	0.009	3.2	0.03
Riboflavin	2.0	0.05	3.0	0.09	1.5	0.12	2.7	0.014	5.6	0.06
B_6	3.0	0.07	1.4	0.04	1.1	0.09	2.2	0.011	2.5	0.03
Nicotinic acid	3.9	0.09	2.2	0.07	1.0	0.08	1.6	0.008	2.2	0.02
R, B_6	2.0	0.05	2.2	0.07	2.4	0.19	1.8	0.009	3.3	0.03
B_1, R	3.1	0.07	3.9	0.12	2.3	0.18	1.1	0.006	4.7	0.05
B_1, B_6	3.6	0.09	2.5	0.07	2.3	0.18	1.7	0.009	6.5	0.07
B_1, R, B_6	5.0	0.12	5.3	0.16	1.8	0.14	2.1	0.011	2.2	0.02
B_1, R, B_6, N.A.	6.4	0.15	4.5	0.13	2.3	0.18	2.8	0.014	2.2	0.02
Yeast	5.5	0.13	2.3	0.07	2.9	0.21	6.2	0.031	4.6	0.05

rats lost almost as much weight as the control rats on the no-yeast diet. On B_1 alone they lost less weight; on B_1 plus riboflavin they did much better; on B_1 plus B_6, still better; on B_1 plus riboflavin plus B_6 they almost maintained their weight; and on all 4 vitamins the rats made a small gain, but their weight still fell far short of that of the yeast control group.

During the first 25 to 30 days the rats offered all 4 vitamins grew at almost the same rate as the normal controls, but after this time they stopped gaining weight. It is not astonishing that the weight gains made by the animals closely parallel the caloric consumption of each group.

Endocrine glands. The weights of all the endocrine glands of the control no-yeast group of rats fell far below those of the control yeast group. The loss of body weight of the rats without any components of the vitamin B complex accounted for a good part of the discrepancies in the endocrine weights of the two groups. However, even when the endocrine weights were calculated in relation to body weight, it was found that the weights of thymus, uterus, ovaries and adrenals still fell below those of the yeast controls. The effects produced on the endocrine glands by

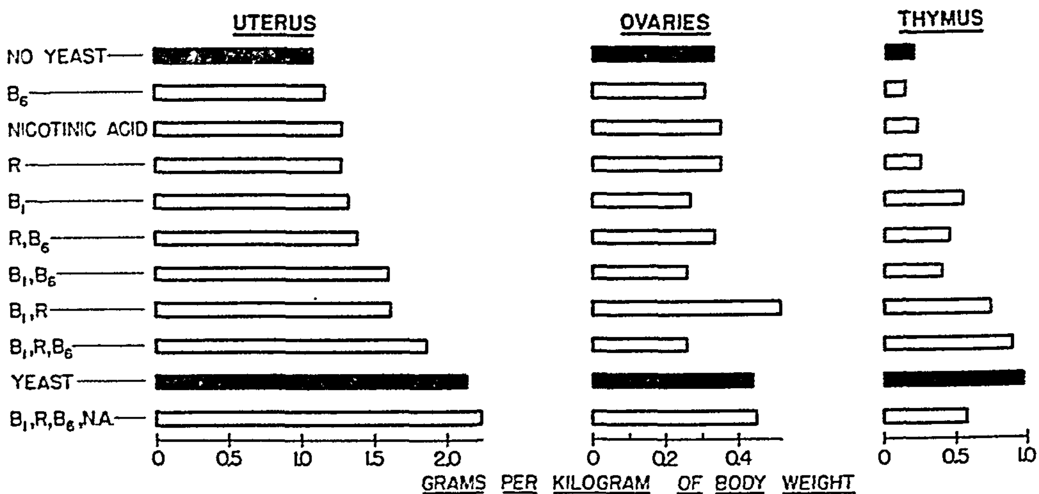

Fig. 3. Graph showing the average weight of the uterus, ovaries and thymus per kilogram body weight for the 2 control (blacked rectangles) and 9 experimental groups of rats.

the various vitamin combinations closely paralleled the effects produced on body weight, caloric intake and the appetite for carbohydrate, fat and protein. Figure 3 summarizes some of the results. It gives the weights of the uterus, ovaries and thymus in grams per kilogram of body weight. For the no-yeast group the uterus averaged 1.1 grams, and for the yeast group, 2.1 grams, or nearly twice as much. The single vitamins had very little effect on the uterus. Here again definite changes depended on combinations which included thiamin chloride. The rats with all 4 components had uteri with weights which averaged slightly above those of the control yeast group (2.2 grams). The ovaries showed similar, however less consistent, effects. The thymus showed the most striking effects. Whereas in the yeast controls the thymus weights averaged only slightly less than 1.0 gram, in the no-yeast controls they averaged only 0.2 gram.

The various combinations of vitamins, especially those including thiamin chloride, progressively increased the thymus weights. The adrenals showed similar effects. However, on the single vitamins, riboflavin and B_6, or on the combination of these two components, the adrenals almost attained the weights of the yeast controls.

The vaginal smear cycles closely paralleled the uterine weights. In the control no-yeast group of rats the vaginal smears showed a dioestrous condition after the 14th day on the diet, while the rats on the full diet had regular 4- to 5-day cycles throughout the 40-day observation period. With all of the single vitamins the regular cycles persisted only about the same length of time as with the no-yeast diet. With the combinations the cycles persisted progressively longer. On all 4 vitamins, 3 rats showed regular cycles throughout the 40-day period; one rat, up to the 33rd day. Clearly, the 4 vitamins maintained the reproductive tract even though they did not fully maintain the entire body.

Discussion. By their appetite, the rats deficient in all components of the vitamin B complex indicated that they were unable to use carbohydrate, and especially protein, but were able to use fat. Biochemical studies have already shown that rats deficient in vitamin B_1 are unable to utilize carbohydrate. The inability to use protein also agrees with the reports of Hartwell (1921, '22, '28), Reader and Drummond (1925), Tscherkes (1926), Cox and Hudson (1929), Bell (1934), and others who showed that a physiological relationship exists between the animal's ability to utilize protein and the amount of vitamin B present in the diet. These results indicated that it is chiefly the number of the then called vitamin B_2 components upon which the protein utilization depends. Our results indicate that thiamin chloride and especially riboflavin play important parts, but that nicotinic acid and B_6 also participate. However, the great discrepancy between the protein appetite of rats which received all 4 of the components and that of rats which received yeast indicated that protein appetite may depend even more on some of the other components, such as pantothenic acid, W factor, adenylic acid, or other ingredients of yeast, such as choline chloride, nucleic acid, etc. The rats further indicated by their appetite that riboflavin may play a part in the utilization of fat. We have not found any biochemical reports which would support this observation.

Thus, from these experiments we see that the higher the number of B vitamins present in the diet the greater is the appetite for carbohydrate and protein and the smaller the appetite for fat, and further, that there exists an inverse relationship between the carbohydrate and fat appetite. Thus far we have invariably found that as one increases the other decreases.

The results also indicated a close dependence of the thymus and the reproductive tract on the vitamin B content of the diet. Here again all

4 components helped to maintain the reproductive tract and the thymus. Singly, if any, they had only a small effect.

The fact that the appetite for calcium and phosphorus solutions increased as more and more vitamins were added may indicate that these vitamins helped the rats to utilize these minerals or they may have only indirectly assisted in growth and maintenance of the skeleton.

Attention may be called again to the fact that under these circumstances, when rats were able to regulate their intake of these various purified substances, in the 40-day observation period no deficiency symptoms appeared. It is possible that in other experiments in which a mixture of regular synthetic or natural food diets was used the appearance of pathological symptoms depended more on harmful effects which resulted from the inability of the animals to increase or decrease their consumption of fat, carbohydrate or protein separately, in relation to the amount of any one component of the B complex eliminated from the diet, rather than from a lack of necessary substances.

SUMMARY

1. A positive control group of 8 young adult rats was given access to our full self-selection diet consisting of: sucrose, olive oil, casein (autoclaved and purified), 5 mineral solutions, cod liver oil and dried baker's yeast. On the selections made these rats gained weight at a normal rate, showed regular 4- to 5-day estrous cycles and normal endocrine glands. Carbohydrate constituted 55.8 per cent of the average diet; fat, 18.4 per cent; and protein, 25.8 per cent.

2. A negative control group of 4 rats was given access to the same substances with the exception of yeast. Thus, their diet lacked all of the B vitamins. They lost weight at once; lost their sex cycle in 14 days; and after 40 days there was a marked atrophy of the endocrine glands, particularly of the thymus, ovaries, uterus and adrenals. Their appetite showed marked changes. They ate little carbohydrate (24.9 per cent), almost no protein (7.5 per cent), and subsisted largely on fat (67.6 per cent).

3. Nine experimental groups of 4 rats each had access to the same basic self-selection diet without yeast, but with crystalline thiamin chloride, riboflavin, nicotinic acid, and vitamin B_6, offered singly or in various combinations. The rats showed an active appetite for each of the vitamins.

4. The ingestion of thiamin chloride particularly stimulated the carbohydrate appetite; riboflavin seemed to have a stimulating effect on the fat appetite; but no one vitamin had an exclusive effect on the appetite for any of the three main foodstuffs. Ingestion of all 4 vitamins increased the carbohydrate appetite almost to normal and decreased the fat

appetite to normal, but failed to increase the protein appetite much past half the normal level; however, the protein appetite was clearly influenced by the intake of thiamin chloride and especially of riboflavin.

5. The ingestion of the 4 vitamins markedly increased the calcium lactate and sodium phosphate appetite.

6. Animals offered all 4 vitamins gained weight for 3 weeks almost at the normal rate; then they lost weight at a moderate rate, but by the end of a 40-day experimental period they had not fallen below their initial weight. The rats on all other combinations of vitamins lost weight steadily. The loss of weight varied almost in inverse proportion to the number of different vitamins. The weight curves closely followed the total caloric intake.

7. The autopsy weights of the uteri, ovaries and thymus indicate that their functioning, too, can be altered by diet. The rats given the 4 vitamin B components came closest to having glands of normal weight (as compared to the yeast controls).

8. Thus, through regulation of the vitamin B components of the diet we were able within large limits to increase or decrease the carbohydrate, fat and protein appetite almost at will. The experiments bring further evidence to show that thiamin chloride seems to serve as a basis for the action of the other components of the vitamin B complex.

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THE INFLUENCE OF STIMULUS STRENGTH AND DURATION ON THE RESPONSES FROM CORTICAL STIMULATION THROUGH IMPLANTED ELECTRODES[1]

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Moruzzi (1939) has lately studied responses to cortical stimulation in non-narcotized rabbits, though the brain was exposed. Such acute experiments upon man and animals have usually involved exposure of the brain with restraint or recent anesthesia, factors which affect the results. With electrodes permanently implanted in the skull and making contact with the brain it is possible to apply stimuli to the cortex without many of the unnatural factors accompanying acute exploration (Clark and Ward, 1937). It is true that with implanted electrodes only a few points on any one animal can be stimulated, but this factor can be offset by the use of more animals. And there is the extra advantage that in a single animal the same point can be stimulated repeatedly on the same and on successive days under special conditions for a period of days or weeks. Lubinska and Konorski (1939) have seen the advantages of a similar procedure.

Since the beginning of the use of implanted electrodes in this laboratory a number of animals, including cats, monkeys, dogs and goats, have been devoted to such experiments. The observations to be reported here have been taken from the recorded results of the numerous stimuli applied through these electrodes. A 60 cycle sine-wave current was the source of stimulation.

Method. The electrodes employed were unipolar. The stigmatic electrode was a piece of silver or platinum wire, sealed in a glass rod of appropriate size with one end rounded off so that the wire was flush with the surface which was to be in contact with the brain. At the other end of the wire a drop of solder furnished a surface for a pressure contact for one lead wire from the stimulating apparatus. The glass-covered wire was inserted into a segment of rubber tubing and this into a stainless steel jacket. The jacket had tapering threads on one end and was screwed into

[1] The work on which this paper is based was aided in its earlier phases by a grant from the Division of Medical Sciences of the Rockefeller Foundation, and during the last six months by a grant from the John and Mary R. Markle Foundation.

a small trephined hole in the skull after the exposed dura mater had been removed. This metal jacket in contact with bone, subcutaneous tissue and skin acted as the indifferent electrode. A special clip allowed the lead wires to be attached and detached rapidly to the electrode.

The stimulating device was similar to one described by Myers (1936) and consisted of a small transformer attached to the 110-volt lighting circuit, with a potentiometer connected so as to allow variation in the strength of the stimulating current and with a voltmeter to record it. A timing device was constructed which allowed duplication of stimuli as to length.

Results. Usually stimulation of animals was delayed until the day following implantation of the electrode, and then to determine a threshold a stimulus thought to be too weak to produce much effect was applied. Following this at intervals of a minute or more slightly stronger stimuli were applied until one was accompanied by definite movement. Record was kept of the duration, strength and time of application of each stimulus, as well as a description of its effects on the animal. The threshold stimulus for a point was arbitrarily chosen as that stimulus which produced visible movement only during the time of its application.

With the apparatus as it was arranged, the average threshold selected for stimulating a point on the motor area was 1 to 2 volts applied for 2 to 4 seconds. Such a threshold remained fairly constant during an experiment in the same animal though it might vary from day to day. On any particular day the threshold could be varied by experimental procedures, and by some physiological factors.

Most stimuli were applied for two seconds (or more) in order that the movements produced would have time to show themselves in completed form during the time of stimulus. For example, the movements most often used in these experiments were batting or digging of the fore paw. These movements, easily obtained from the anterior margin of the cat's motor cortex (Ward and Clark, 1935), were rhythmic, and with them it was possible to observe the repetition of a cycle instead of just a sustained contraction.

After establishing a threshold, experiments were done with variations in the strength and duration of stimuli as well as the interval between successive stimuli. The threshold stimulus was used as a basis for comparison and could be repeated whenever necessary as a check. By raising the voltage or prolonging the stimulus an after discharge could be made to appear. Such after discharges varied all the way from mere prolongation for a few seconds of movement of the affected part of the animal to a complete epileptic attack lasting one or more minutes.

In order to illustrate the effects of stimulation graphic representations of some of the results are shown in accompanying figures 1 and 2. In the

diagrams each horizontal bar represents one stimulus and its effects. The length of the bar to the left of O represents volts. The length of bar to the

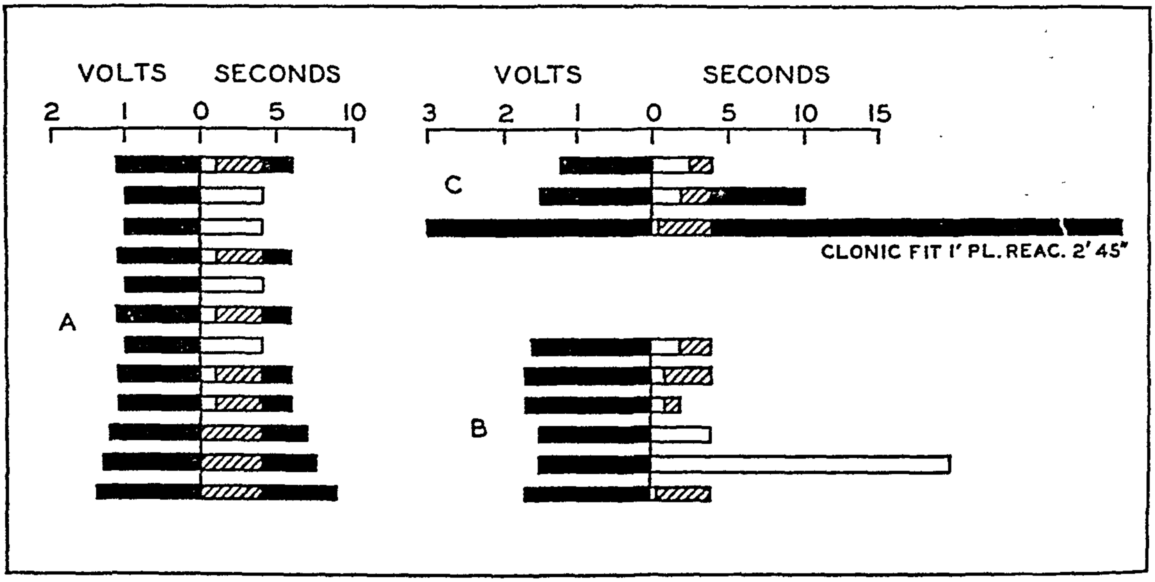

Fig. 1. Graphic representation of a few stimuli and responses to cortical stimulation in two cats. For description see text. In each cat the electrode was on the left anterior sigmoid gyrus, the movement produced being digging with the right fore paw.

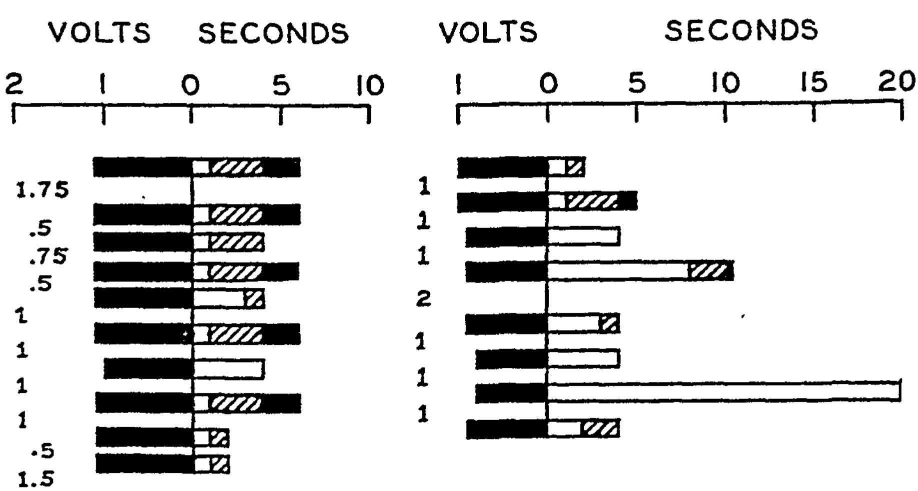

Fig. 2. Graphic representation, described in the text, showing the effect of varying intervals between stimuli on the responses to cortical stimulation in a cat. The electrode and movement were the same as in figure 1A. The numbers in the column to the left represent the intervals in minutes between successive stimuli.

right of O represents time. The duration of the stimulus in seconds is represented to the right of O in the portion of the bar that is not solid black. When movement appears during the stimulus the bar is filled

with oblique lines. The solid black part of the bar to the right represents the duration of movement following completion of the stimulus, i.e., after discharge. The white part of the bar to the right before the appearance of motion represents the period of latency. The interval between successive stimuli is two minutes, unless otherwise indicated by a number to the left of the spaces between bars.

The effects from stimuli of equal duration but different strengths. When sufficient time for recovery was allowed between successive stimuli the latency of the response and the magnitude and duration of the movements resulting from stimuli of constant length varied with the strength of the stimulus employed.

In figure 1A, it can be seen that a stimulus to the motor area of a cat's brain of 1 volt for 4 seconds gave no response, but 1.1 volts for 4 seconds gave after about 1 second latency, a response which lasted 2 seconds beyond the stimulus. When the stimulus was increased to 1.2, 1.3 and 1.4 volts (the last three bars) the latency was abolished and the after effect prolonged.

The effects from stimuli varied in duration and strength. The responses to stimuli varied with the duration of the stimulus as well as the strength. In figure 1B, (the same electrode as in 1A but on another day) it can be seen that a stimulus of 1.6 volts for 4 seconds gave a response after 2 seconds' latency but with no after effect, while 1.7 volts for 4 seconds gave a response after a shorter latency. The same strength stimulus (1.7 volts) for 2 seconds gave a response with the same latency and no after effect. One and five-tenths volts gave no response with a stimulus of 4 seconds or even after a stimulus 20 seconds in length. Two minutes after the latter stimulus, however, a stimulus of 1.7 volts gave a typical response following a very brief latent period.

Figure 1C shows the results of three stimuli at two minute intervals in another animal. The first stimulus, 1.2 volts for 4 seconds, was followed by a response after a latency of 2.5 seconds. The next stimulus, 1.5 volts for 4 seconds, after a shorter latent period gave a response with a 6 second after effect. The third stimulus, 3 volts for 4 seconds, resulted in a clonic fit lasting a minute, with a loss of placing reactions for a total of $2\frac{3}{4}$ minutes.

The influence of varying the interval between successive stimuli. Figure 2 shows a succession of stimuli through the same electrode that was used in figure 1A and B but with variations in the intervals between stimuli. (The second column follows the first after 1.5 min.) The intervals between stimuli are indicated to the left of each space, but are also proportionately represented by the width of the space between successive bars. It can be seen that a standard response was obtained with a stimulus of 1.1 volts for 4 seconds in which a latency of 1 second and an after effect of 2 seconds accompanied the response, provided at least 0.75 minute elapsed between

stimuli. With a stimulus of 1 volt a lessened response was obtained. With 0.9 volt for 4 seconds no response was obtained after a minute interval, although a 10 second stimulus (at 0.9 volt) gave a response after an 8 second latency with also a slight after effect. A stimulus of 0.9 volt for 4 seconds gave a response when 2 minutes elapsed between stimuli; while a stimulus of 0.8 volt gave no response with 4 seconds' or 20 seconds' stimulation following a one minute interval.

The effects of stimuli of constant strength and duration applied after short equal intervals. While an interval of a minute or more between stimuli was necessary to allow the reproduction of the original effects of successive threshold stimuli (as defined), the application of a stimulus for the first

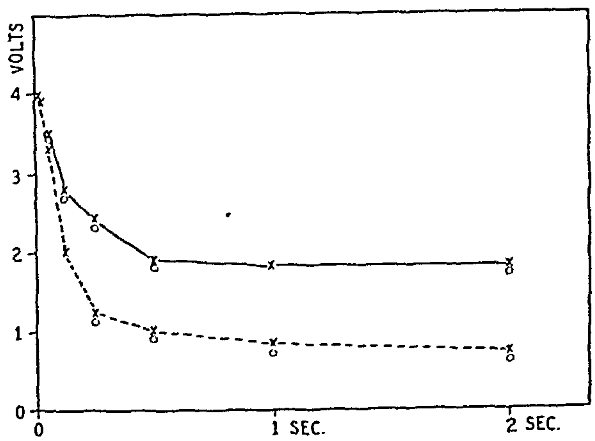

Fig. 3. Graph showing the influence upon threshold strength of altering the duration of the stimulus. The duration of stimuli is expressed in seconds on the horizontal axis, the strength in volts on the vertical. The two curves were obtained from experiments on different cats. The points marked with *X* indicate the stimulus strength and duration necessary for threshold response. The points marked *O* indicate the nearest preceding ineffective stimulus.

two seconds out of every fifteen for several minutes resulted in the establishment of a new level of response. The first response in such a series of stimuli was like that to previous stimuli of threshold strength, but the responses to the next few stimuli were progressively diminished until a constant but lessened response following each stimulus successively applied. Such a series of stimuli could be continued for ten minutes or more without abolishing the response, or appreciably altering its magnitude after the new level was established.

The effects of decreasing the duration and increasing the voltage of successive stimuli. Another type of experiment was done to determine the effect of increasing the voltage but decreasing the duration of successive stimuli in a series (fig. 3). After the establishment of a threshold, a stimulus of threshold strength was applied for half the established time. If the re-

sponse did not appear, the next stimulus was increased in strength by 0.1 volt and this process repeated with successive stimuli until a response did appear. Then the duration of the following stimulus was arbitrarily cut in half and so on. With brief stimuli it was not possible to produce responses of sufficient duration to be described as entirely similar to those following stimuli several seconds in length, but it was possible to see evidence of responses, even though they were mere twitches of the affected muscles; and it was evident that the strength of each new threshold stimulus increased as its time of action was decreased.

DISCUSSION. From examination of the figures showing samples of series of stimuli to the cerebral cortex through implanted electrodes it can be seen that the effects of successive applications of a given stimulus of near threshold value were reasonably constant when sufficient time between stimuli was allowed for recovery.

If a stimulus was applied which was increased beyond the previously established threshold by raising the voltage or lengthening the time of application, until an after discharge appeared, then the time of recovery was prolonged. Such an after effect was constant in its duration for the particular stimulus which provoked it, and could be repeated subsequently when the conditions were the same. The duration of after effect depended upon the amount of increase in stimulus strength or duration, or both, over that of the selected threshold as well as upon the immediately preceding history of the experiment. With only slight increase of stimulus above threshold strength the magnitude of the muscular response was greater; if the response was rhythmic the rate of the rhythm might be quickened. At a strength of stimulus two or three times that of threshold the animal would be thrown into a generalized epileptic seizure lasting several minutes, which spread in its beginning like the "march" of a Jacksonian convulsion. If a generalized epileptic attack was provoked phenomena associated with it might be present for five to ten minutes or more and recovery was much delayed. During this period of recovery stimuli of threshold value would fail to produce the usual response, and the stimulus ordinarily sufficient to provoke a fit might fail entirely, or be followed by an abbreviated after discharge, the duration of which varied with the time since the preceding convulsion.

The same type of response as that following threshold stimulus was produced by a slight lowering of the voltage and appropriate lengthening of its time of action, but in this case the movements often appeared a short while after the beginning of the stimulus. The response was therefore preceded by a latent period. The length of this latent period has been shown by Ward (1938) to vary for a given stimulus dependent upon such factors as: the position of the animal's head, the position of the responding part; the just preceding sensory experience of the animal; its

state of wakefulness; the integrity of reflex paths, etc. Lubinska and Konorski (1939) demonstrated similar alterations in the latency in varying physiological states.

If the strength of successive stimuli was progressively lowered by small amounts below that of the threshold, it was necessary at each level tested to prolong the duration in proportion to produce visible responses. Not far below threshold a limit was reached at which point no response could be obtained with a stimulus of indefinite duration (figs. 1B and 2). The division between an effective and an ineffective stimulus was rather sharp. With a prolonged weak but effective stimulus, say of 15 to 20 seconds' duration, the visible movements often ceased before the end of the stimulus.

After cessation of visible movements provoked by a given stimulus the placing reactions in an affected limb might be negative for a while longer. Furthermore, following a stimulus too weak to produce visible movement even after 20 to 30 seconds' application, the placing reactions of the proper extremity might be abolished for a while. This may be correlated with the observations of Moruzzi on the evidence of bioelectric effects from the cortex following subliminal stimuli.

As the stimuli in our experiments were relatively long in duration we were constantly dealing with the phenomenon of facilitation, which has been studied repeatedly. This is illustrated, for example, by the period of latency preceding responses to subthreshold stimuli, the appearance of a response some time after the onset of the repetitive stimulus being one evidence of facilitation. Such a latency was termed by Cooper and Denny-Brown (1927) "summation period" to distinguish it from the latent period of nerve fibers. These authors state that facilitation may be avoided by spacing stimuli one minute apart. In the present experiments it was the rule to allow two minutes to elapse between stimulations, unless a briefer time was indicated as a part of the experiment.

In these experiments we have also been aware of the factor for extinction described by Dusser de Barenne and McCulloch (1937). They observed that the optimum interval for extinction in the monkey in the waking state was 4 seconds, and that it was prolonged by anesthesia. It is possible that the conditions of our experiments are sufficiently different to result in different manifestations of this phenomenon. The diminished responses obtained from the stimulation after the first of a series when the stimuli were applied for the first 2 seconds out of each 15 are apparently evidence of the operation of this factor. It might be questioned whether the diminished response to stimulation in the longer period following an epileptic seizure is evidence of a more prolonged operation of the factor for extinction, but the time limits of the chart of the above authors (1939, fig. 15) might allow this in part. During the period immediately follow-

ing an induced epileptic seizure, when the placing reactions are negative it is obvious that the cortex is not functioning normally. We have pointed out (1938) how this temporary state resembles the permanent condition produced by removal of the motor area. Even after the placing reactions have returned, however, for a while stimulation of the same point on the cortex with the same stimulus which just previously provoked an epileptic seizure will either not produce a fit, or will produce one of less severity. This state of diminished response to strong stimulation may last several hours.

The actual values of the stimuli of threshold strength are no doubt not indicated by the figures recording the voltages at the ends of the electrodes we have used, since the electrodes rest on the pia mater in a bath of cerebrospinal fluid; and in addition to the pia there is the superstructure of the cortex between the stigmatic electrode and the cortical elements (of the deeper layers, Dusser de Barenne, 1934) which are activated. However, relative strengths of successive stimuli are indicated and the stimulation is measurable in terms which can be applied to other conditions. The same apparatus has been used by Dr. Cobb Pilcher in exploring exposed human brains and similar threshold values obtained, though with anesthesia thresholds were higher. This similarity of thresholds in different species recalls the experiment in which Leyton and Sherrington (1917) connected the exposed brains of a cat, a macaque, and a chimpanzee in series with a stimulator and found that motor responses occurred at approximately the same threshold in each.

We wish to acknowledge the aid in conducting some of the experiments on which this paper is based of Mr. J. Thomas Payne and Mr. Fred C. Cowden.

SUMMARY

Cortical stimulation through implanted electrodes allows the study of responses in unanesthetized and unrestrained animals, in which with constant conditions responses to stimuli can be repeated and predicted. With stimuli of near threshold strength the response varies quantitatively with the duration and strength of the stimulus. An interval of one minute or more is necessary for recovery from the effects of a stimulus of near threshold strength.

Subthreshold stimuli followed by no visible movement may be followed by a temporary loss of placing reactions in the appropriate contralateral limb.

The duration of responses beyond the time of stimulus allows the production of after effects up to and including complete epileptic seizures.

The duration of such after discharges is predictable when the factors for variation are properly considered. The recovery period is prolonged following such after effects.

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THE GRADATION OF THE INTENSITY OF INSPIRATORY CONTRACTIONS[1,2]

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It has been generally agreed that the mechanical energy employed in eupneic breathing is provided mostly, and sometimes entirely, by the inspiratory muscles. Similar conclusions are now warranted for the hyperpnea of oxygen want for if the expiratory muscles are active during eupnea their contractions are often seen to weaken or actually to disappear as pulmonary ventilation increases (Brown, Atkinson and Gesell, 1939). Thus the burden of breathing may fall entirely to the inspiratory muscles even during conditions of respiratory distress. Not only must these muscles provide greater energy to overcome the greater distortion of the torso and lungs during each inspiratory act but they must supply increasing potential energy for the expulsion of the air as well. The adjustment of inspiratory contraction thus promises to be the most important single factor of respiratory control. Our present paper deals specifically with the basic nature of the graded adjustment of these contractions to the mechanical requirements of the inspiratory act and with the sudden relaxation of their activity during the expiratory period (see fig. 1 and legend).

Two points are already clear from the studies of Adrian and Bronk (1928). Strength of muscular contraction is determined primarily by two factors: 1, the frequency of muscle fiber twitch, and 2, the number of active muscle units engaged. Our problem then resolves itself into specific phases. First, the relative importance of twitch frequency and of the number of units participating, for that may suggest the underlying integrative mechanism. Second, the precise manner in which these basic tools of gradation are used, and third, a hypothetical consideration of the nervous mechanism effecting this use.

Two obvious assumptions may be made. Mechanical energy may be liberated in a suddenly developing rectangular block sufficient to accomplish the required inspiration of air in the time allotted to the inspiratory act. With that method inspiration would tend to be jerky. Or

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mechanical energy may be liberated to match the growing requirements of increasing resistance to pulmonary inflation. That method should produce a smoother inspiration.

In their studies on the action potentials of the phrenic nerve in the rabbit, Adrian and Bronk saw no indications of increasing strength of contraction as each *individual* inspiration developed. They state that

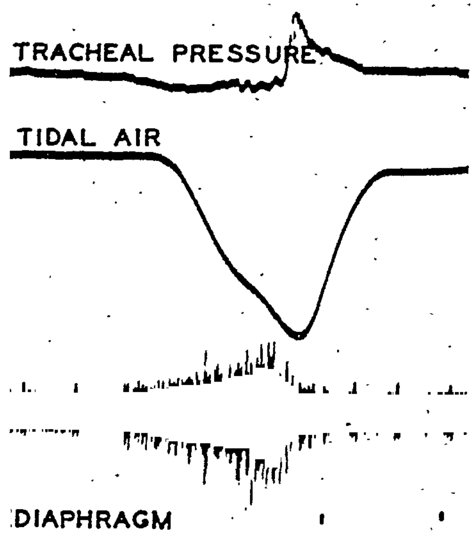

Fig. 1. An inspiratory fusillade recorded with widely separated floating electrodes placed on the surface of the diaphragm of the dog. The upper record shows changes in tracheal pressure during the inspiratory and expiratory phase. The middle or tidal air record shows the velocity of movement of the inspired and expired air. Due to the weight of the spirometer there is a large time lag. The peak of the electrogram and the trough of the tidal air tracing should coincide.

The relatively even descent of the spirometer tracing and the relatively constant inspiratory pressure indicate that the air is inspired at a relatively even velocity despite a continuously increasing resistance to the expansion of the lungs. The rising electrogram indicates a progressively increasing strength of inspiratory contraction. The abrupt emptying of the lungs, indicated by the sudden rise of the tidal air record and the sudden development of positive intratracheal pressure, the "expiratory puff," shows the suddenness of the expiratory act. The sudden relaxation of the inspiratory muscles revealed in the electrogram conforms with a powerful passive expiration.

each nerve fiber discharges at uniform frequencies throughout each inspiration (between 20 to 30 per sec.) and that accession of new muscle units is missing. Thus strength of diaphragmatic contraction remains uniform throughout inspiration. They "looked for evidence of accession of fresh fibers as contraction develops by comparing the duration of the single fiber discharge with that of the whole nerve," but "found no clear evidence of single fiber discharge being any shorter than that of the whole nerve."

Forcible contractions produced by clamping the trachea yielded twitch frequencies of 50 to 80 per second which led these authors to conclude that "variations in frequency of discharge between the limits of 20 to 80 a second are excellently adapted for producing contractions of graded intensity without bringing fresh fibers into play."

The results of Adrian and Bronk (1929) on the flexion reflex were very much the same as those on the diaphragm. They state "that grading of the contraction appears to be due mainly to this change in frequency, for there is little evidence of changes in the number of neurons in action." In the extension reflex on the other hand they found "that many fresh neurons come into play as contraction develops." Bronk and Ferguson (1935), reporting on the internal intercostal muscles, conclude that "Variations in depth of intercostal respiration are a result of variations in frequency of discharge from the individual nerve cells, the duration of their discharge and the number of nerve cells in action." These results are similar to those of Gesell (1936) and Gesell and White (1937) on the internal and external intercostal muscles in which a slowly augmenting triangular pattern of activity was described. Rijlant (1937) is of the same opinion as Adrian and Bronk, that the contractions of the diaphragm are of the explosive type in the cat, rabbit and the dog.

Granting the validity of these results, one is confronted with the query—why should the gradation of contraction of inspiratory muscles differ in such important details? Why should the relatively unimportant intercostal muscles deliver a nicely graded contraction capable of overcoming a progressively increasing resistance, and the more important diaphragm deliver energy in an explosive rectangular block? Since the activity patterns of respiratory contractions reveal the details of the end effect of the nervous integration of the respiratory act, and tell us in such precise form what we wish to explain, it seemed most desirable to extend the existing studies on the nature of respiratory contractions.

Results. On moving highly selective electrodes indiscriminately from one point to another, one soon finds with the aid of a loud speaker alone, that many fibers are inactive during eupnea, that some fibers twitch throughout the entire period of inspiration while others twitch but once or twice at the very end of inspiration, that the remaining active fibers contract variable periods of time and fill in the intervening gap, that twitching of any muscle unit begins with a relatively low frequency and accelerates as inspiration progresses and that cessation of twitching is relatively abrupt. Due to the differences of sound (as heard with the loud speaker) produced by individual muscle fibers, it is possible to distinguish one series of twitches superimposed upon another in every stage of the inspiratory act. Such results are common to the internal and external intercostal muscles and the diaphragm, thus indicating that ac-

celeration of frequency of twitch and recruitment of new units are *universally* employed in the gradation of inspiratory contractions.

Widely separated, instead of closely approximated electrodes, will pick up simultaneously the potentials of individual muscle units in every stage of recruitment such as illustrated in records 1, 2, 3, 4, 5 and 6 of figure 3 and combine them into a heterogeneous fusillade. No single set of discrete

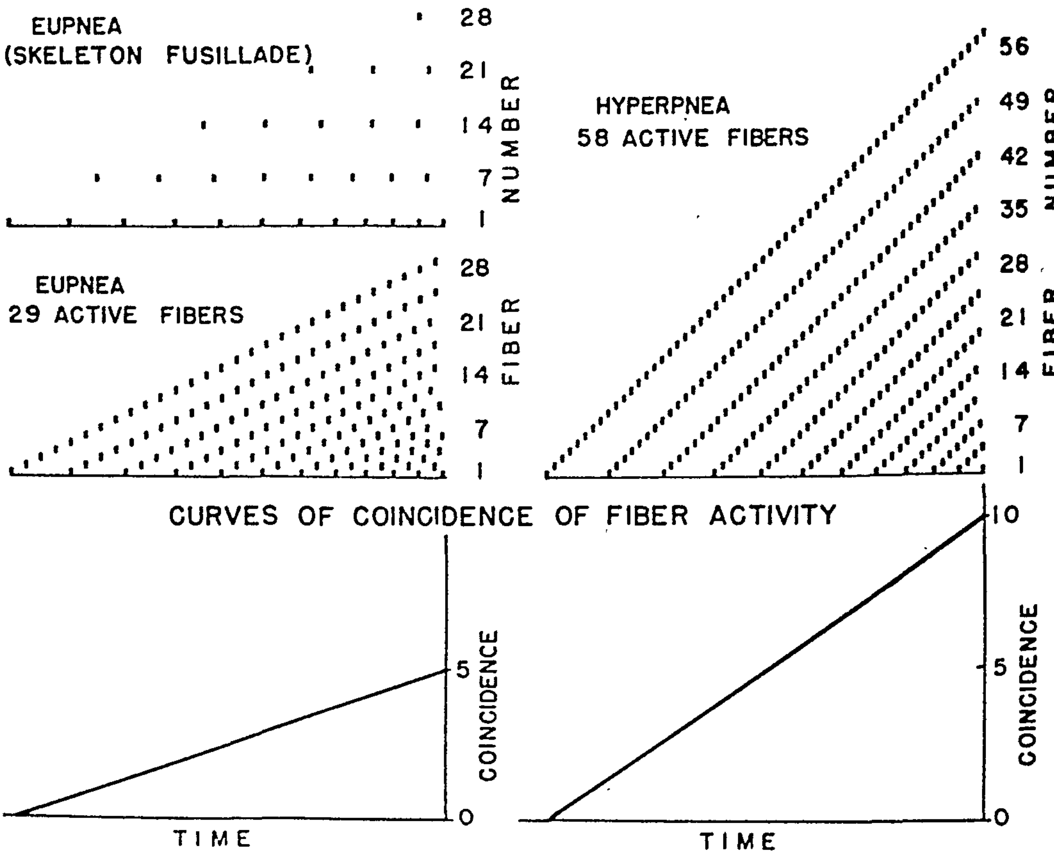

Fig. 2. A schematic representation of the use of recruitment and acceleration of muscle fiber twitch in the adjustment of inspiratory contractions to the mechanical requirements of shallow and deep breathing. For explanation see text. If inspiration is sufficiently strong and prolonged the initially active fibers may reach their maximum twitch frequency an appreciable interval before the termination of the fusillade (see fig. 3).

potentials of any single muscle unit can be heard but rather a mixture of asynchronous potentials of slowly increasing and suddenly decreasing volume, agreeing with the graphic record of the diaphragmatic fusillade of figure 1.

The necessarily increasing incidence of potentials as one unit after another is recruited we believe explains the increasing volume of sound and the triangular shape of the electrical fusillade in figure 1. This point is

schematically illustrated in figure 2, where 29 muscle units were arbitrarily chosen as participating in the hypothetical eupneic fusillade at the left. Muscle unit no. 1, at the bottom of the schema, is the first to contract. It goes through its normal course of acceleration from left to right. Unit

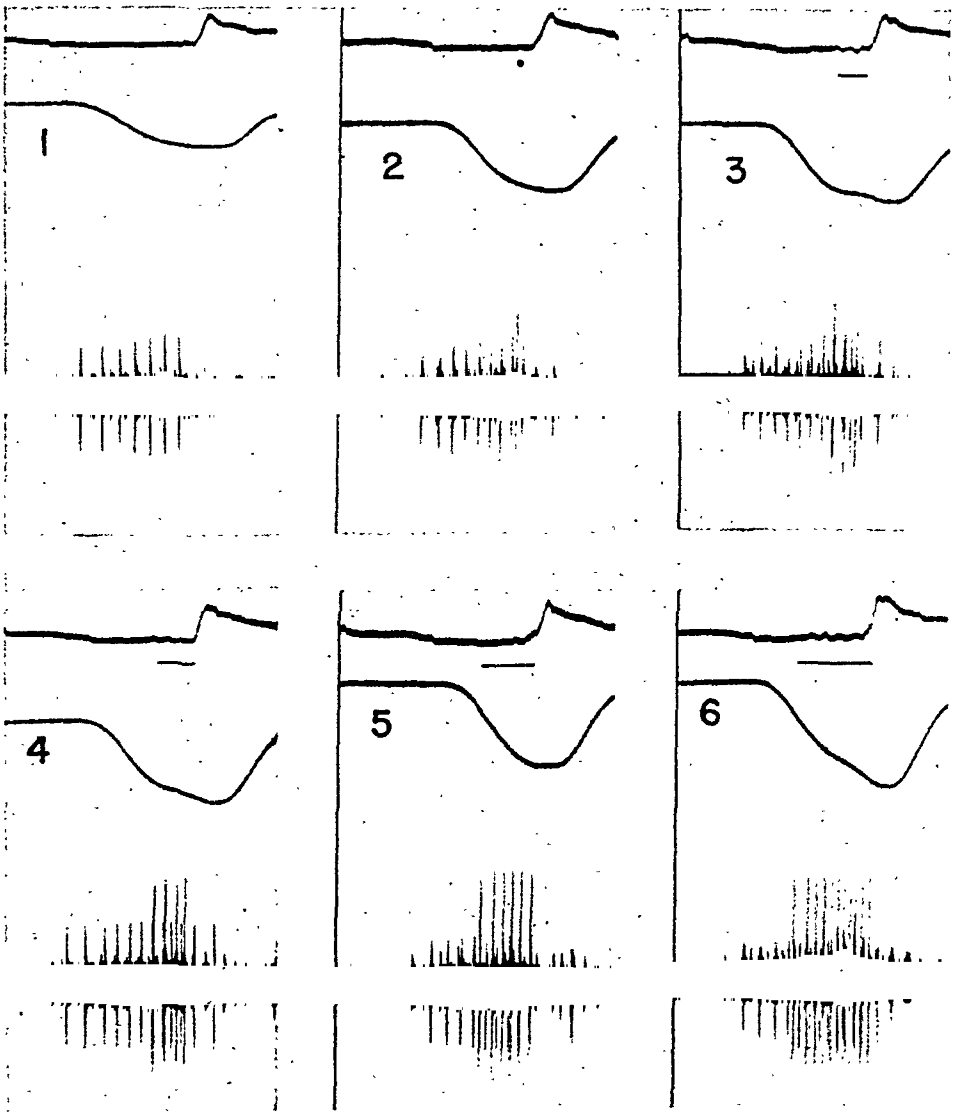

Fig. 3. Actual records of the activity of two muscle units of the internal intercostal muscle during a progressively increasing chemical stimulation resulting from rebreathing into a confined space. Acceleration of twitch frequency, recruitment, inspiratory encroachment, and increasing twitch number are illustrated (see text).

no. 2 contracts next, and unit no. 3 follows closely on unit no. 2, etc. Each muscle unit begins its activity at the minimum frequency of twitch and accelerates, in this schema, at the standard pace up to the end of inspiration. The muscle units which are recruited late in the phase of inspira-

tion, therefore, fail to acquire the final high frequency of twitch of unit no. 1. This is more clearly seen in the upper skeleton fusillade in which units 1, 7, 14, 21 and 28 are shown by themselves. On moving a vertical line along the complete fusillade, the incidence of potentials will be seen to increase from left to right as more active units are recruited and as the frequency of twitch of each unit increases. One potential thus adds to the other and raises the electrical record as already noted in figure 1. The photographic record rises, unlike the smoothed schematic curve of incidence of fiber activity in figure 2, in a serrated fashion, agreeing with an actual varying incidence of overlapping potentials which must occur in any purely asynchronous mechanism such as muscular contraction. We have been unable to find groups of synchronized activity of muscle units corresponding to the frequency of twitch such as described by Adrian and Bronk (1928) and Rijlant (1937). On that point we are in agreement with Wyss (1939). Since each muscle fiber twitch adds its quota of mechanical energy to that of other contracting fibers, as it adds its incident potentials, the rising shadow of the electrical fusillade becomes an index of the strength of muscular contraction.

In addition to the automatic increase of intensity of each eupneic inspiratory contraction necessary to overcome the physical resistance to breathing under uniform respiratory demands, there must also be a means of controlling the depth of inspiration to meet augmented demands such as oxygen lack or carbon dioxide excess. As figure 3 shows, the gradation of inspiratory contractions meeting an increasing chemical stimulation is no different in principle from that employed in meeting increasing physical resistance during each inspiratory act. In this experiment the dog was made to rebreathe a restricted volume of air. As records 1, 2, 3, 4, 5 and 6 show, there is an orderly change in the details of contraction as the chemical stimulation mounts, and the depth of breathing increases. Muscle unit no. 1 begins with 7 twitches in respiration no. 1. In breath 2 the number of twitches is increased to 9. In breaths 3, 4, 5 and 6 the twitches have reached a maximum steady number of 12. In the mean time fiber no. 2 has begun to contract, with but a single twitch at the end of inspiration no. 2. In breaths 3, 4 and 5 it twitches 3, 4 and 6 times respectively, and in breath 6 there are 11 twitches in all. As the number of twitches increases, activity begins earlier and earlier in the inspiratory phase. This advancement of activity is indicated in figure 3 by the horizontal line under the tracheal pressure record.

The schematic fusillades of figure 2 will indicate more clearly the mechanisms underlying the change from eupnea to hyperpnea. Twenty-nine additional active muscle units recruited into activity by a hypothetical increased chemical stimulation, have been added to the original 29 units of the hypothetical eupneic fusillade. Initial activity of succeeding units,

therefore, follows in more rapid succession and thus increases the strength of inspiration at a greater pace than normal. The units initially active during eupneic breathing now contract a greater number of times per inspiration and, therefore, contribute an increased amount of mechanical energy to the inspiratory act, thus units 7, 14, 21 and 28 instead of contracting 8, 5, 3 and 1 times respectively now contract 10, 8, 6 and 5 times. The newly recruited units 30 to 56 now top off the contraction. A count of the total number of twitches for the complete fusillades shows 150 for the eupneic inspiration and 282 for the hyperpnea. Doubling the number of fibers, therefore, falls a little short of doubling the sum total of twitches. That is due to the fact that a larger number of fibers fail to reach a high frequency of twitch during hyperpnea.

The advancement of activity into the earlier stages of inspiration as chemical stimulation increases is most rapid in each new unit immediately after its recruitment into service; see for example figure 4 in which the recorded potentials of three muscle fibers during a progressive rebreathing hyperpnea were transposed under a single respiratory schema. When unit no. 1 first recorded it was already twitching 5 times per inspiration and consequently the initial twitch of this series had advanced well into the earlier stages of inspiration. But as hyperpnea increased initial activity encroached still more upon the early phase of contraction. Finally each twitch occurred at the approximate onset of inspiration. Unit no. 2 fortunately recruited with a single twitch and, therefore, shows the initial rapid "inspiratory encroachment." The lower curve shows the inspiratory encroachment of initial activity of unit no. 3.

If the energy liberated by a single twitch of a single muscle fiber is tentatively considered as the unit of muscular energy, the number of such energy units contributed by individual muscle fibers in the course of an increasing ventilation from increased chemical stimulation is worthy of consideration. It is readily seen in figure 4 that as chemical stimulation increases, each muscle fiber contributes an increasing number of twitches to each inspiration. That is due to a prolongation of the period of twitching and to an acceleration of the frequency of twitch. The course which this increase commonly follows is shown in figure 5. The number of twitches per inspiration of three individual muscle fibers plotted against intensity of stimulation (breath number after the beginning of rebreathing) on the abscissae is seen to increase rapidly immediately after each muscle fiber is recruited into activity, then more and more slowly and finally it tends to strike a relatively uniform level though often this level is not fully attained under the conditions of our experiments. The curve is very similar to that already described for inspiratory encroachment in figure 4. A significant fact is that the curve of twitch number appears to have a similar contour at all intensities of chemical stimulation and that newly activated

fibers continue to be successively recruited after the early activated units have already reached or approximated their maximum activity. In

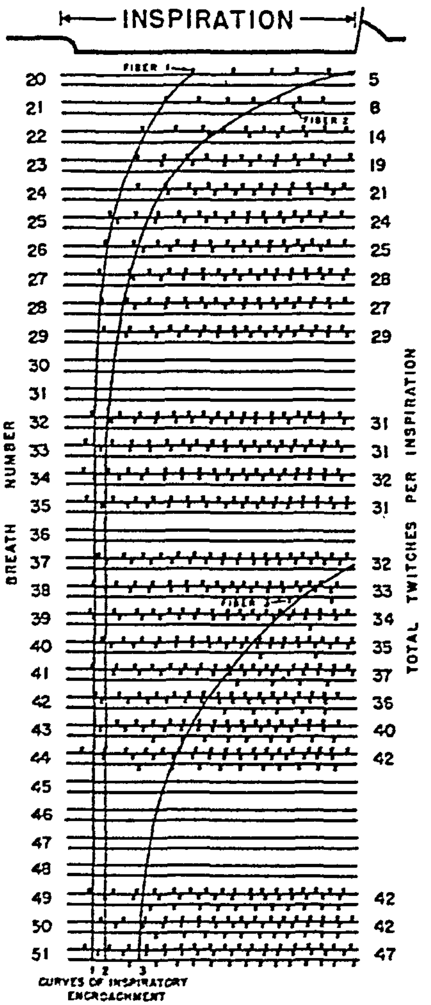

Fig. 4

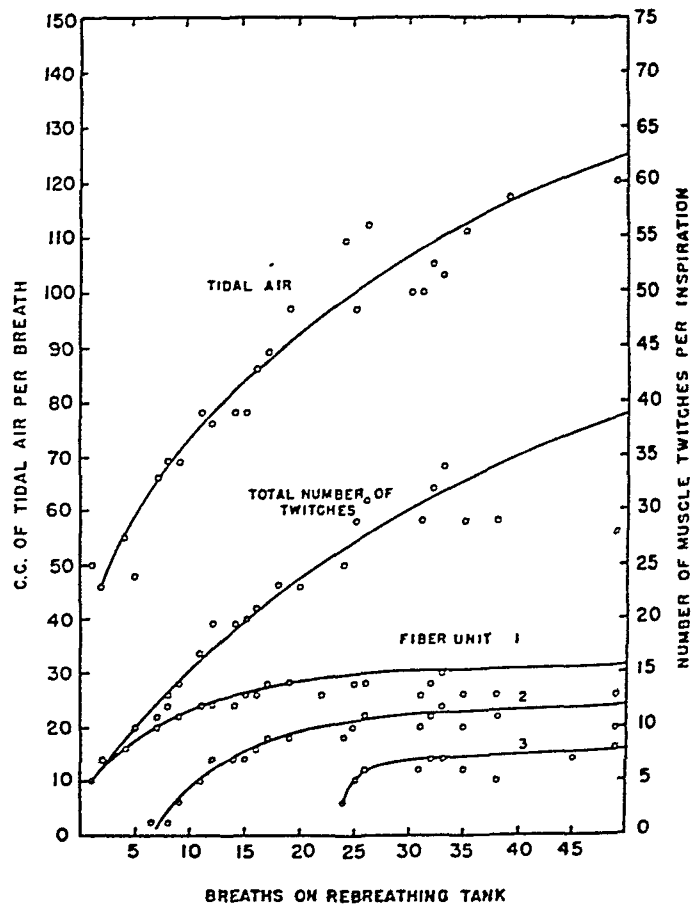

Fig. 5

Fig. 4. A transposition of electrograms of three muscle units under one inspiratory schema during an increasing hyperpnea (breaths 20 to 51 after the beginning of rebreathing into a confined space). The curves show the course of inspiratory encroachment of the initial twitch of three muscle units. The individual records show the gradual increase of twitch number and twitch frequency with increasing chemical stimulation.

Fig. 5. The lower three curves show the relation of the number of twitches per inspiration for three muscle units during an increasing chemical stimulation. The figures on the abscissae indicate the breath number after the beginning of rebreathing into a confined space. Note the lack of agreement of these curves with that of tidal air or strength of inspiratory contraction. There is better agreement between the tidal air curve and that of the total number of twitches.

figure 5 muscle unit no. 3 begins its activity when unit no. 1 is nearly at its maximum response. Obviously the number of twitches contributed by

any *single* muscle unit cannot be regarded as an index of total muscle activity.

Changes in the maximum twitch frequency, that is, the highest frequency occurring during the course of inspiration, are illustrated in figure 6. As was seen for the curves of inspiratory encroachment and of twitch number the curve of twitch frequency in each individual fiber rises rapidly immediately after its recruitment and increasingly slower thereafter, and, as was true for inspiratory encroachment and twitch number, new muscle units

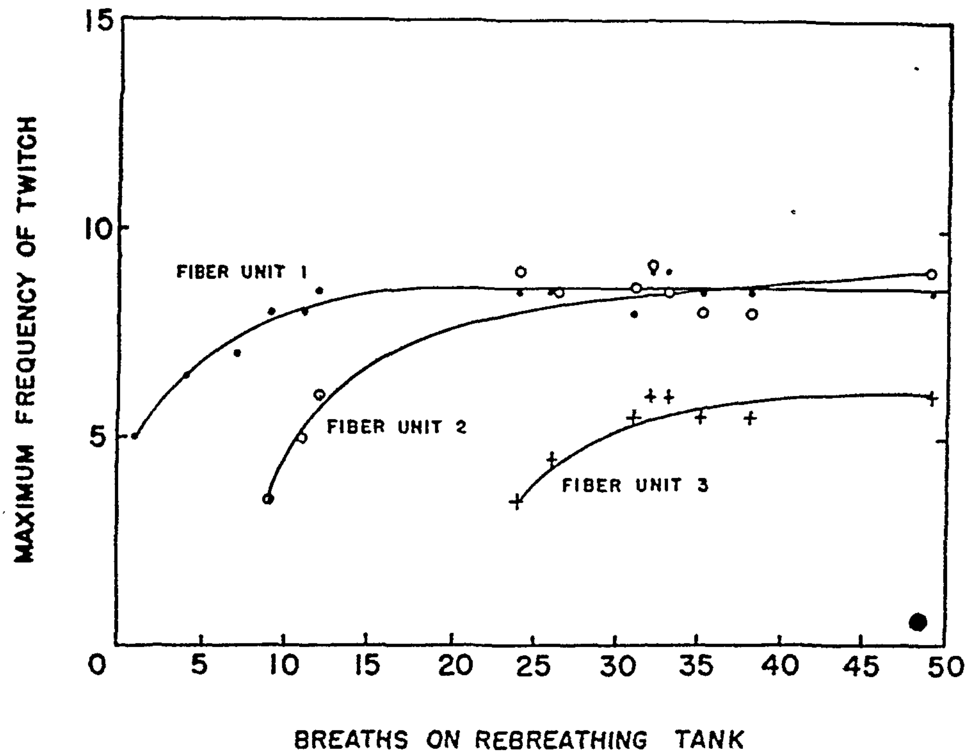

Fig. 6. Maximum twitch frequency of three muscle units during a progressively increasing chemical stimulation. These results and those of figure 5 were obtained from the same observation. Note the low frequency of twitch.

successively recruited run through their complete frequency change after earlier activated units have already reached their maximum frequency of twitch. Maximum twitch frequency of any single unit cannot, therefore, represent the intensity of the contraction of the muscle as a whole as originally suggested by Adrian and Bronk. Because the curves of inspiratory encroachment, of twitch number, and of twitch frequency all have the same shape at all stages of intensity of contraction none can indicate the change of intensity of contraction. We must, therefore, look for another index.

The most important index is in all probability the sum total of twitches or mechanical energy units liberated by all those fibers participating in

contraction. That is suggested by the comparison of the curves in figures 5 and 6. Only when the sum total of twitches of all three units is plotted against intensity of chemical stimulation is there a semblance of agreement with strength of inspiratory contraction as represented by the curve of tidal air (see fig. 5).

It is true that tetanic contraction of a muscle may quadruple the tension developed by a single twitch. This has been noted by Creed, Denny-Brown, Eccles, Liddell and Sherrington (1932) and by Cooper and Eccles (1930) in skeletal muscle and by Adrian and Bronk in the negative intratracheal pressures developed by the contraction of the diaphragm. Yet none of these findings are contrary to the concept that the sum total of individual fiber twitches is the factor determining the strength of contraction, for the total number of individual twitches would vary directly as the frequency of stimulation. Furthermore a control of strength of contraction based purely on a frequency mechanism would yield but a small degree of gradation as compared with the possibilities of recruitment. For instance, there can be no question of the fact that fewer and fewer of the muscle units of the diaphragm contract as an animal is made progressively more hypocapnic. Conversely, it is reasonable to assume that all units are ultimately called into activity by an intense hypercapnia. Admitting that the diaphragm has thousands of individual muscle units capable of the finest gradation of recruitment, it follows that the possibilities of the gradation of intensity of contraction through the mechanism of recruitment may be thousands of times greater than that of gradation by frequency alone. To one accustomed to hearing the amplified potentials of respiratory contractions there can be no doubt of the validity of this conclusion.

Frequency of twitch remains surprisingly low even during strong chemical stimulation, from 10 to 30 in the intercostals and somewhat higher in the diaphragm. Such an arrangement combined with a smoothly graded recruitment to call in extra energy as it is needed must be a most effective protection against fatigue, not only in the muscle, but throughout the central nervous system as well. No single unit is allowed to set a devastating pace leading to an early collapse. Instead new units are called in to support those already contracting. For these reasons we questioned the principle of alternate rest and activity of individual units. And to test this idea more critically we have attempted to learn how long a single muscle fiber would contract without retiring in favor of another. Such periods may run four hours or more. On the basis of such findings one is tempted to suggest that certain muscle fibers, for example those activated by cell 1 of figure 8, are destined to discharge with each inspiration throughout life, during each inspiratory act, whereas those connected with the underlying cells are destined to lead a relatively inactive existence con-

tracting only under emergencies requiring more powerful inspiratory contractions.

It, therefore, seems most significant in this connection that the increase in tension developed by increasing frequency of stimulation of muscle is greatest in the lower twitch frequencies. According to the curves of Adrian and Bronk (1928) and of Cooper and Eccles (1930) the relation is roughly linear in the twitch frequency range normal to each individual muscle. At higher frequencies the strength of contraction is increased relatively

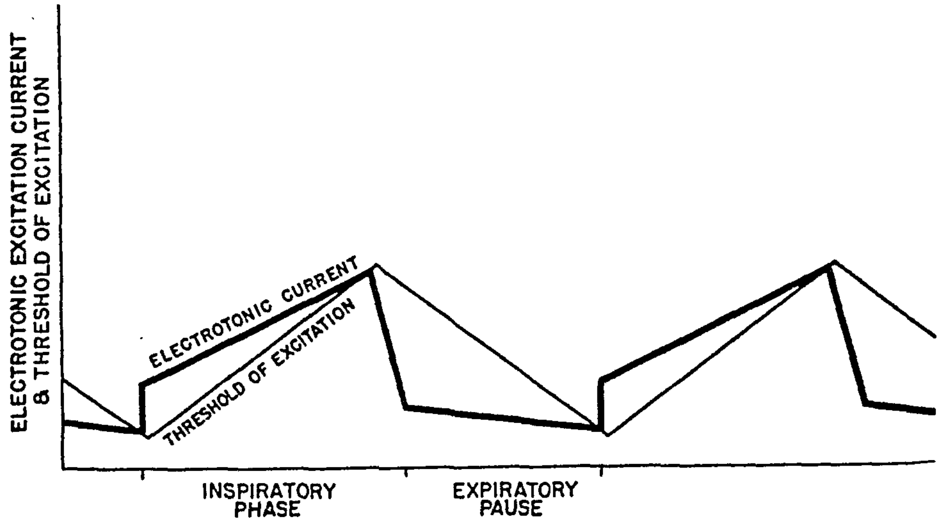

Fig. 7. An hypothetical schema of acceleration and interruption of nerve cell discharge based on the augmenting action of signals returning by the recurrent collaterals.

When the nerve cell begins to discharge the electronic excitation current is conceived to rise due to the progressively increasing number of recurrent signals. The resulting increasing frequency of discharge exhausts the cell and the threshold of excitation of the axon hillock rises. When it is greater than the excitation current the cell stops firing. The electrotonic excitation current then falls abruptly due to the absence of recurrent signals and ultimately more slowly. As the cell recovers during this period of inactivity the threshold of excitation likewise falls and in time the cell fires once more.

little. Thus it should follow that recruitment of one fiber twitch after another in an augmenting contraction adds a relatively uniform amount of energy.

It is well to add that we have attempted to emphasize principles rather than details. Nevertheless completeness requires a brief mention of some variations of response. In certain observations, for example, the curves of twitch number and maximum frequency rose less abruptly at the outset than those described. Some curves rose after the break at a somewhat steeper pitch than did others. We gained the impression that such dif-

ferences held more for the diaphragm than for the intercostals although the results on these two sets of muscles were in general the same.[3] In some experiments it was noted that the curve of twitch frequency of the diaphragm rose longer and broke higher than that of the intercostals. There is some evidence that the machine-like regularity of performance

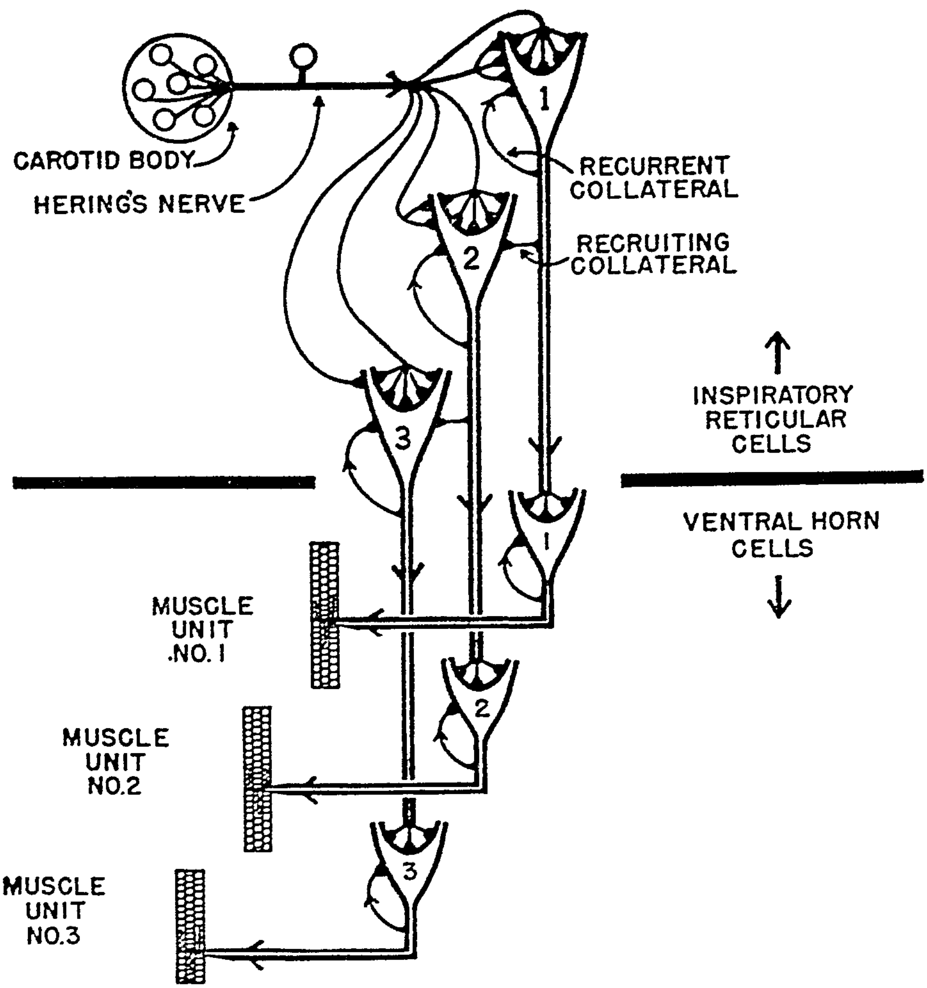

Fig. 8. An hypothetical neuro architectural arrangement of nerve cells designed as a possible explanation of an orderly recruitment of nerve cell activity out of the subliminal fringe.

of the inspiratory muscles is modified by proprioceptive impulses for when asphyxia was produced by clamping of the trachea instead of by rebreathing the curves rose in a different manner. Such difference is probably the

[3] It should be noted that our observations on the diaphragm were made during pneumothorax in which conditions are decidedly abnormal.

result of the addition of one respiratory drive to another. Since our conclusions are based on a large number of findings we have no reason to believe that such variations as do occur contradict the general principles of gradation of contraction set forth above under increasing chemical drive.

THEORETICAL. The rhythmical recurrence of the slowly augmenting pattern of discharge of the inspiratory center during curari paralysis and the complete abolition of periodic afferent signals leads to the conclusion that such activity arises from steady drives acting upon some mechanism peculiar to the inspiratory center (Gesell, Atkinson and Brown, 1940). The regular uninterrupted series of discharges produced by a steady polarizing current in the iron nerve model of Lillie (1923), the Nitella (Osterhout and Hill, 1930), the heart, and the isolated nerve fiber (Adrian, 1930) lend support to an electrotonic theory of nerve cell activity (Gesell, 1939a, 1939b, 1940). The rhythmical interruption of the discharges of an isolated nerve responding to its own current of injury (Adrian, 1930) signifies that secondary as well as primary rhythms may develop in exceedingly simple structures. Our theory of a single possible mechanism of a slowly augmenting nerve cell discharge in brief is this. An electrotonic current flowing in the cell from the dendrites to the axon hillock discharges the cell at its point of exit. This current, assumed to arise from an inherent metabolic gradient and local negativities established by impinging signals tends to fire the cell at a uniform frequency. Due however to signals returning by recurrent collaterals the cell reëxcites itself progressively to increasing activity until it suddenly fails from temporary exhaustion. During the resulting period of inactivity the cell again recovers its normal excitability to its own electrotonic current and then repeats the cycle as suggested in figure 7.

Recruitment of new active units would in turn depend upon simple motor interconnections (fig. 8), between the inspiratory reticular cells comparable to those originally described by Retzius and Linnhosek (cited by Cajal, 1909). According to our hypothetical schema, the activity of one cell is transmitted to a second resting cell, thereby bringing that cell into activity. Acceleration of frequency of discharge and recruitment thus become most intimately related. Cell 1, possessing the lowest threshold of excitation, sets off the inspiratory discharge. It at once dispatches signals to cell 2 (whose excitation current is just below threshold) and momentarily sets this cell discharging. As cell 1 accelerates, it in turn tends to accelerate cell 2. Cell 2 in turn connected with cell 3 tends to activate that cell accordingly. When cell 1 ceases to fire it withdraws its signals from cell 2. Thus all underlying cells cease firing. Since cell 1 ceases abruptly all underlying cells stop discharging in a like manner which explains the sudden weakening of the inspiratory fusillade.

SUMMARY AND CONCLUSIONS

The relatively great importance of the inspiratory act in the mechanics and in the nervous integration of breathing called for a further study of the mode of its gradation.

Since inspiratory contractions produce a progressively increasing distortion of the lungs and torso the amount of energy required to continue the act must vary with the degree of pulmonary inflation.

This energy is found to be supplied in a smoothly graded manner suitable for meeting the increasing mechanical requirements as the lungs expand.

The adjustment of energy is accomplished in two ways, by a progressive addition of newly activated units to the initially weak contraction and by an increased frequency of twitch of all participating fibers as inspiration advances.

Each newly recruited unit begins contracting at its minimum twitch frequence which accelerates as inspiration progresses. Those units recruited early in the phase of inspiration, therefore, attain the highest frequency and deliver the greatest number of twitches.

The gradation of inspiratory contractions during progressive hyperpnea from oxygen lack or carbon dioxide excess is in principle the same as that employed to meet the ordinary increasing mechanical requirements of a eupneic inspiration. Increased depth of inspiration is attained by a combination of recruitment of new muscle units and an intensified activity of those units already engaged. As chemical stimulation increases, initial activity of individual muscle units moves up into the early phases of inspiration thus increasing their number of twitches and their maximum twitch frequency.

The energy liberated by a single twitch of a single muscle fiber is adopted as the unit of mechanical energy of muscular contraction and the sum total of the individual twitches of all participating fibers is regarded as the main factor determining the strength or depth of inspiration. The number of twitches and the frequency of twitch of individual muscle units is not necessarily an indication of the strength of contraction of the muscle as a whole.

Central integrative mechanisms of periodicity and of gradation of the activity of the inspiratory half center are proposed.

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THE DUAL EXCITATORY ACTION OF THE VAGAL STRETCH REFLEX[1]

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The emphasis on the inspiratory inhibitory action of the pulmonary vagi has been characteristic of virtually all modern views of respiratory control (Hering and Breuer, 1868; Gad, 1880; Head, 1889, and Adrian, 1933). To us, however, it has seemed more consistent to regard the vagal stretch reflex as an excitatory phenomenon, both inspiratory and expiratory, in which the associated inhibitions of the opposing centers are but secondary manifestations of the excitatory action (Gesell, 1939). The simple experiments here described present evidence supporting such hypothesis.

METHOD. A dog, anesthetized with morphine and urethane, is connected with a rebreathing tank arranged for the absorption of expired carbon dioxide and for the registration of tidal air excursions. The spirometer employed in these experiments is specially constructed to allow an artificial increase of intrapulmonary pressure while recording pulmonary ventilation. Two encircling bands placed around the depilated torso register the changes in circumference at the mid thoracic and mid abdominal levels (Gesell and Moyer, 1935). Segmental and combined respiratory response are then recorded on smoked paper during normal barometric conditions, during increased intrapulmonary pressure and during increased intrapulmonary pressure plus double vagal block. Downstroke in the respiratory records corresponds to an increasing circumference of the chest and abdomen and to a filling of the lungs.

RESULTS. The general trend of results is illustrated in figure 1. In this observation normal breathing is recorded for a period of thirty seconds, after which the lungs are inflated above normal volume by loading the spirometer. During the period of pulmonary inflation which follows, the cervical vagus nerves are twice blocked and deblocked at separated intervals and finally the spirometer is unloaded and the animal allowed to breathe normally once more. The first noticeable effect of the expanded condition of the lungs and torso is an immediate cessation of rhythmic breathing. Since this well-known response is absent or almost missing

[1] This study was supported by a grant from the Rockefeller Foundation.

when the vagus nerves are blocked or severed (note the acceleration of breathing during both vagal blocks), it is generally regarded as due to an inspiratory inhibitory action arising in the vagal stretch reflex.

The next visible respiratory response, not as well known as the reflex stoppage of breathing, is a slowly developing expiratory activity. This effect was emphasized by Hering and Breuer when they found a slowly rising intratracheal pressure following an artificially increased pulmonary volume. In our experiments increased expiratory activity is evidenced by a decreased circumference of the abdomen opposing the distending pressure.

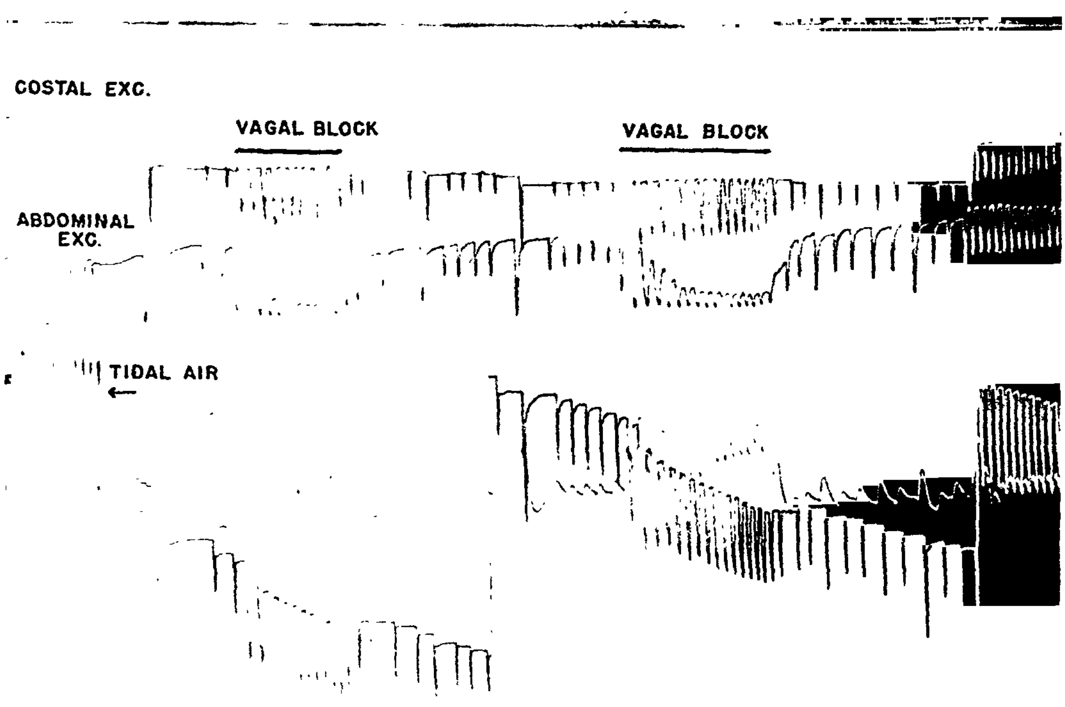

Fig. 1. Effects of exaggeration of the vagal stretch reflex produced by a sustained superinflation of the lungs. For discussion see text.

The simultaneous "inhibition" of the inspiratory act and the increased activation of expiratory muscles considered by themselves are in full agreement with the original Selbsteuerung theory of Hering and Breuer (1868). But the powerful respiratory cycle breaking through the so called inspiratory apnea is most difficult to reconcile with an inspiratory *inhibitory* action of the vagi. The extremely rapid filling of the lungs of this and the next two inspirations to follow cannot be due to the simple mechanical effects of the increased pressure of the air seeking entrance into the lungs. Were that factor important, each expiration should be much *slower* than normal for the air is then leaving the lungs against a greater resistance than normal. On the contrary these expirations are exceedingly rapid. Fur-

thermore, double vagal block during increased intrapulmonary pressure produces striking changes in the respiratory records. Breathing assumes a more swinging gait. Despite a greatly increased depth of breath the velocity of both inspiration and expiration is actually diminished. It therefore seems highly probable that vagal block removes two powerful excitatory effects—one exerted on the inspiratory half center and the other exerted on the expiratory half center. It is well to recall that even Gad (1880), who accepted only an inspiratory inhibitory action of the vagus nerve, noted a greater velocity of inspiratory contractions when the vagus nerves were intact. More recently this same slowing of inspiration after vagotomy has been described by Nicholson and Brezin (1937), and Gesell, Steffensen and Brookhart (1937), and others.

Another striking effect of vagal block during exaggerated reflex vagal activity is the tremendous reduction of expiratory activity in the abdominal segment. The abdomen tends to assume an inspiratory position upon which weakened expiratory contractions are superimposed. This is regarded as conclusive evidence of the powerful excitatory action which the vagi may exert upon the abdominal musculature during excessive inflation of the lungs. Evidence for a similarly increased vagal expiratory activity, however, is missing in the thoracic records for there are no clear indications of constriction of the chest during increased intrapulmonary pressure. As a matter of fact there is a small constriction of the chest during vagal block while the abdominal circumference is increasing. Whether this opposite effect upon the chest is of reflexogenic origin or whether it is due to the increased activity of the diaphragm sucking the chest walls in as the diaphragm descends and pushes the abdomen out is not clear from these experiments and must be decided by action potential studies.

The dual excitatory action of the vagal stretch reflex mentioned above is not without precedent in other forms of respiratory stimulation; e.g., Gesell and White (1938) have shown that the effects of momentary excitation of the chemoceptors of the carotid body vary with the time that such excitation occurs in relation to the respiratory cycle. Thus cyanide injected into the carotid artery, timed to reach the carotid body during the phase of inspiration, will strengthen that particular inspiratory act, while injection timed to stimulate the chemoceptors during the expiratory phase of breathing will intensify that particular expiration. Since there are no apparent reasons for dividing the chemoceptors into inspiratory and expiratory groups there are no apparent objections to assuming that each chemoceptor possesses double central connections, one group of endings synapsing in the inspiratory half center and the other group synapsing in the expiratory half center (see fig. 2). The number of endings in the half centers would thus determine only the relative strength of inspiratory and expiratory stimulating action of the chemoceptors. The same prin-

ciple may be applied to any form of sensory drive. Since stretching of the lungs is found to stimulate both inspiratory and expiratory muscles, as does the discharging carotid body, there is need of assuming but one active set of proprioceptive end organs in the vagal stretch reflex and that such end organs possess dual connections at the center. Since many of these end organs never cease firing (Adrian, 1933) but discharge with a waxing

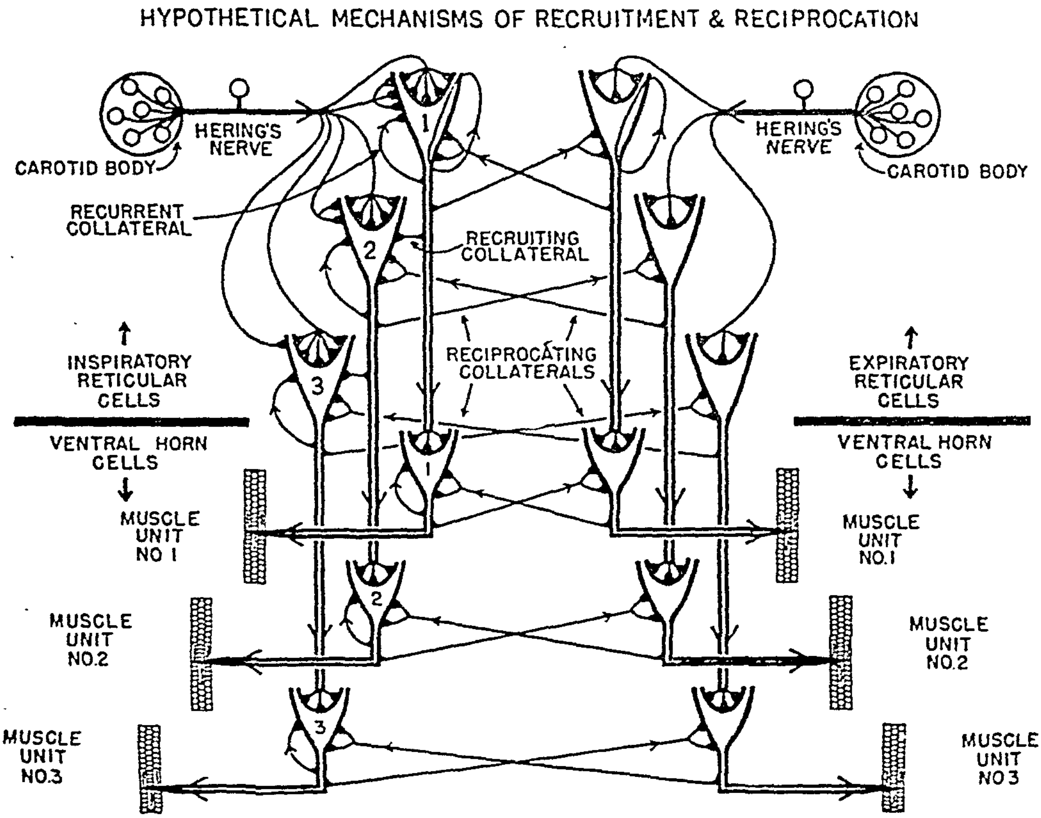

Fig. 2. A schematic representation of the dual connections of the chemoceptors and of the reciprocating connections between half-centers which permit a steady chemoreflex drive to produce a rhythmic alternating inspiratory and expiratory activity. Similar inspiratory and expiratory connections of the individual vagal stretch receptors (see text) should provide a means for shifting the periodic vagal drive along with the steady chemical drive from one half-center to the other with each changing phase of respiratory activity.

frequency during the inspiratory phase and with a waning frequency during the expiratory phase, they are theoretically capable of driving both acts of respiration in a manner similar to the chemoceptors. This we believe is possible through the intervention of reciprocating connections between half centers.

The electrotonic theory of nerve cell discharge offers an extremely simple mechanism of reciprocal inhibition and of alternating activity of half-

centers (see fig. 2). While the inspiratory reticular cells 1, 2 and 3 are discharging they are assumed to dispatch signals via their reciprocating collaterals to the inhibitory poles of the expiratory reticular cells. Theoretically this opposes the prevailing excitatory current in the expiratory cells and holds their discharge in check. Consequently those signals impinging on the inspiratory half center during the normal filling of the lungs fit in with the phase of inspiration and, therefore, strengthen and speed the inspiratory discharge. On the other hand, those signals simultaneously impinging upon the expiratory half center produce only local synaptic action because the negativity they establish is effectively opposed by the reciprocal inhibition exerted by the inspiratory half center. This condition prevails so long as the inspiratory cells discharge, but once their activity ceases, reciprocal inhibition, or local negativity at the inhibitory pole of the expiratory cells is withdrawn. Only then do the excitatory signals of the vagal endings impinging upon the expiratory cells force these cells to discharge. Conversely the expiratory discharges hold the inspiratory center in check.

Since expiratory activity is importantly determined by the number of vagal signals impinging on the expiratory half center during the expiratory phase it is necessary to recognize that the number of these signals diminishes as the lungs collapse. In contrast to inspiratory contraction, expiratory contractions should, therefore, be strong at the outset and weak at the close as they actually are in a sensitive reflex preparation. Accordingly, reciprocal inhibition of the inspiratory center must weaken as expiration progresses. Since expiratory activity, as witnessed by muscular contraction, is relatively weak or even missing under normal conditions there is relatively little or no restraint upon the development of new inspiratory discharges. When, however, the vagal stretch reflex is intensified by a super inflated condition of the lungs, such as seen in figure 1, the resulting expiratory activity becomes a most important regulatory factor. Heightened reciprocal inhibition of the inspiratory half center holds the inspiratory cells in check until the growing chemical stimulation, central and reflex, increases the electrotonic excitation current above threshold.

Granting that the strength of the current leaving the axon hillock determines the degree of nerve cell activity, a sudden withdrawal of negativity at the inhibitory pole should have excitatory action equal to that of added negativity at the excitatory pole. On that basis half centers should not only exert a reciprocal inhibition on each other but also derive a reciprocal excitation from each other. Such dual reciprocating relationships suggest a tangible explanation of the phenomenon of rebound illustrated in figure 1. It will be seen that the sudden and powerful inspiratory contraction interrupting the initial apnea changes most abruptly into an equally sudden and powerful expiratory contraction. The great strength of the expiratory

contraction, we believe, is a rebound effect explained by a *sudden* withdrawal of a powerful inhibition (or negativity at the inhibitory poles) from the expiratory half center at the very end of inspiration. Immediate and sudden withdrawal of negativity is regarded as important in rebound due to the shortness of the local after-synaptic action continuing in the immediate vicinity of the individual synapses. Thus a sudden and deep inspiration is matched with an equally sudden and deep expiration whereas a more shallow but sudden inspiration (see following 2 respirations) is followed by an equally sudden and shallow expiration.

The irregularity of depth of breathing during super vagal activity such as is seen during the period of pulmonary inflation has been a consistent effect and is readily removed by vagal block. This uneveness of depth seems unrelated to a varying frequency of breathing for a deep breath may occur after a short as well as after a longer expiratory pause. We are inclined to attribute the variations to an uneven intensification of two phenomena. The intensification of the inspiratory discharge resulting from the augmented vagal drive should by itself *increase* the depth of breathing whereas the resulting hastening of exhaustion of the inspiratory half-center should cause a premature cessation of the inspiratory discharge and *decrease* the depth of breathing. This is but an extension of the theory of interruption of the normal eupneic inspiration (Gesell, Atkinson and Brown, 1940) in which a rapidly increasing excitation threshold overtakes a more slowly increasing electrotonic excitation current and thus cuts off the discharge. Since elimination of vagal action eliminates an important factor accelerating each individual inspiration it allows the discharge to develop at a slower rate (see fig. 1) and thus reduces the exhaustion factor. As a result the inspiratory discharges reach a greater and more uniform intensity which is reflected in deep and more uniform breathing.

SUMMARY AND CONCLUSIONS

Increased intrapulmonary pressure was found to produce several important respiratory effects throwing light upon the nature of the vagal stretch reflex.

1. The well-known reduced frequency of breathing.
2. A prolonged and increasing constriction of the abdomen.
3. An increased velocity of the inspiration and expiration of air.

Double vagal block during exaggerated vagal stretch reflex activity abolished abdominal constriction and decreased the velocity of both inspiration and expiration while at the same time it increased the depth of breathing.

It is, therefore, suggested that the vagal stretch reflex is an excitatory phenomenon in which each stretch proprioceptive ending drives both the inspiratory and expiratory half centers by virtue of dual connections at

the center. Coördinated alternate reflex activation of the two half centers is dependent upon reciprocating interconnections.

Vagal inhibition of the inspiratory half center is regarded as a secondary response to vagal excitation of the expiratory half center and vice versa. Thus a reduced frequency of breathing produced by an increased volume of the lungs is explained by an increased activity of the expiratory half center exerting a more powerful reciprocal inhibition of the inspiratory half center.

A mechanism is proposed to explain the nature of vagal action and of "rebound."

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ON THE ORIGIN OF THE EXPIRATORY ACTIVITY PATTERNS[1]

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Three types of respiratory activity patterns are now well established for the dog: 1, the slowly augmenting and rapidly waning; 2, the steady state, and 3, the rapidly augmenting and slowly waning (Gesell and White, 1938; Gesell, Magee and Bricker, 1940; Gesell, Atkinson and Brown, 1940). The first is associated with the inspiratory phase of breathing, the second and the third with the expiratory phase. The slowly augmenting pattern in which activity builds up gradually and subsides abruptly is well adapted to meet the increasing resistance of the lungs and torso during inspiration and of allowing a rapid passive emptying of the lungs during the expiratory period. Passive recoil of the lungs and torso is in addition commonly associated with either a steady state or a rapidly augmenting and slowly waning contraction of the expiratory muscles. The mechanical value of the steady state *contractions* to the movement of air must however be questioned for they so often begin after the lungs have already collapsed to their expiratory volume. On the other hand, their timely *relaxations*, accommodating the following inspiration of air, have definite mechanical value in the inspiratory act. It is, therefore, inadvisable to classify the steady state contraction as purely respiratory for it undoubtedly has a joint function of which the maintenance of visceral posture may be of equal or even greater importance. The rapidly augmenting and slowly waning expiratory contraction is, however, better adjusted to assist the expulsion of air. Its great initial power occurring at the very close of inspiration is conceivably designed to bring pressure on the rapidly receding lungs of passive expiration.

Since elimination of the rhythmic respiratory proprioceptive signals by curari paralysis plus temporary cessation of artificial ventilation does not eliminate the slowly augmenting inspiratory discharges (Gesell, Atkinson and Brown, 1940) this pattern of activity is attributed to the ability of the inspiratory half center to react to a steady form of stimulation with periodic discharges of the slowly augmenting type. In the absence of equally positive evidence on the origin of the expiratory activity patterns

[1] This study was supported by a grant from the Rockefeller Foundation.

a more tentative suggestion was offered in which the steady state discharge was regarded as the basic central pattern of expiratory activity (it, too, was not abolished by curari) and the rapidly augmenting and slowly waning discharge a reflexogenic modification of the steady state discharge. It was pointed out that the action of a powerful but waning discharge of the vagal stretch receptors upon the expiratory half center beginning with the expiratory discharge might explain the origin of the rapidly augmenting and slowly waning expiratory contraction. Our present experiments deal more directly with this problem.

Methods. Expiratory activity was studied with the aid of muscle action potentials during varying intensities of the vagal stretch reflex, produced by rhythmic artificial inflation of the lungs. The choice of muscle proved to be a most important factor for when the abdominal expiratory muscles were employed results were extremely irregular. It was finally concluded that the uncertainty of response was related to an opposing action of extra vagal reflexes for when the lungs are inflated there is not only a stretching of the receptors in the lungs but in the muscle under study as well. It, therefore, seemed imperative to search for more simple experimental conditions. This finally resulted in the use of the thyroarytenoid muscle which contracts normally during the expiratory period. Since the tracheal cannula is inserted below the larynx, local proprioceptive reflexes produced by inflation of the lungs are avoided and the sensory modifications of expiratory activity become more purely vagal. As a further precaution in the same direction the anterior and lateral chest walls were removed to minimize the effects of proprioceptive signals arising in the thoracic cage. These restrictions, we believe, explain the exceptional uniformity of our results.

Results. The breathing of an artificially ventilated animal, as is well known, tends to fall into phase with the stroke of the pump provided reflex sensitivity is high. For reasons which need not be considered now, either expiration or inspiration may coincide with pulmonary inflation. This dual response proved most helpful in establishing the nature of expiratory activity. In figure 1, for example, expiratory activity, indicated by the electrogram, falls into phase with artificial inflation of the lungs. As the piston descends and the lungs distend, intratracheal pressure rises slowly, and when the expiratory exhaust valve opens and the piston rises the lungs collapse more suddenly and the intratracheal pressure falls abruptly to zero. The correspondence between the frequency of twitch and the rate of rise and fall of respiratory volume, as indicated by the tracheal pressure record, is a most significant point. It recalls the findings of Adrian (1933) on the vagal stretch receptors in which a similar relation of frequency of discharge to lung volume change occurred. Since the response of the thyroarytenoid muscle to lung volume changes disappears

on vagal block it is concluded that the vagal stretch receptors are capable of driving the thyroarytenoid muscle in a precise and machine-like manner. This action is in agreement with views previously proposed of the dual excitatory action of the vagal stretch reflex upon the inspiratory and expiratory half centers (Gesell and Moyer, in press).

It is pertinent that figure 1 was obtained during an apneic condition of the respiratory center produced by excessive pulmonary ventilation (when the pump was stopped the animal failed to breathe), for it emphasizes that vagal activity is capable of eliciting a purely reflex expiratory contraction. While Hering and Breuer were among the first to demonstrate an expiratory action of the vagal stretch reflex they made no distinction

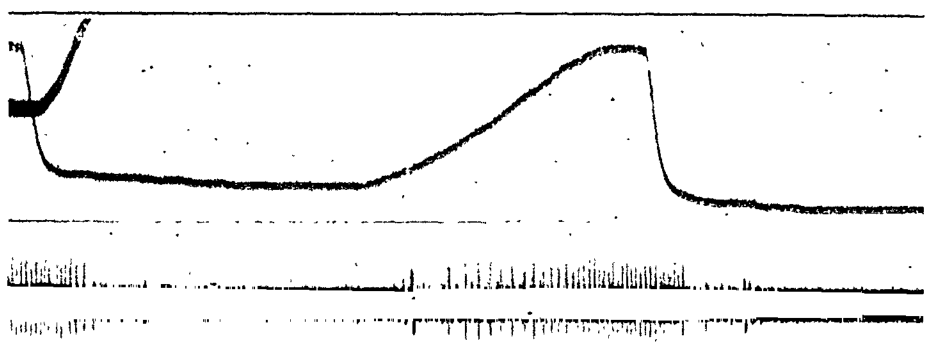

Fig. 1. The electrical response of a single muscle unit of the thyroarytenoid muscle to artificial inflation of the lungs. The gradually rising pressure in the trachea (upper record) indicates a relatively slow and uniform increase of lung volume, while the sudden drop of pressure indicates a more rapid recoil of the lungs. Excepting for the short "after-discharge" following recoil there is a very close relation of frequency of twitch to the prevailing intensity of the vagal stretch reflex.

between a purely reflex and a modified expiratory activity nor were they interested in activity patterns. These points are now considered.

First of all it will help to bear in mind that the increasing vagal activity which occurs during the progressive filling of the lungs in normal inspiration should theoretically produce a progressive intensification of the waxing inherent inspiratory discharge, whereas the decreasing vagal activity occurring during the emptying of the lungs should withdraw stimulation from the expiratory center as expiration progresses and produce a waning expiratory discharge (Gesell, 1940). Such hypothesis agrees with respiratory activity patterns now established. It is, therefore, interesting to note that the expiratory contraction illustrated in figure 1 is contrary to the activity patterns of normal breathing for it is of the slowly augmenting type characteristic of inspiratory activity. But this is readily explained, for we have already shown that the intensity of a purely vagal reflex contraction varies directly with the intensity of discharge of the vagal stretch

receptors. Thus the atypical expiratory pattern is a direct result of the slow augmentation of vagal activity which occurred during the *artificial* inflation of the lungs.

Conversely an artifically created vagal discharge of waning intensity made to impinge upon the expiratory center during the expiratory phase of breathing, should produce a rapidly augmenting and slowly waning pattern of expiratory activity. Such impingement was accomplished in figure 2. Fortunately the frequency of the artificial inflation of the lungs was slow.

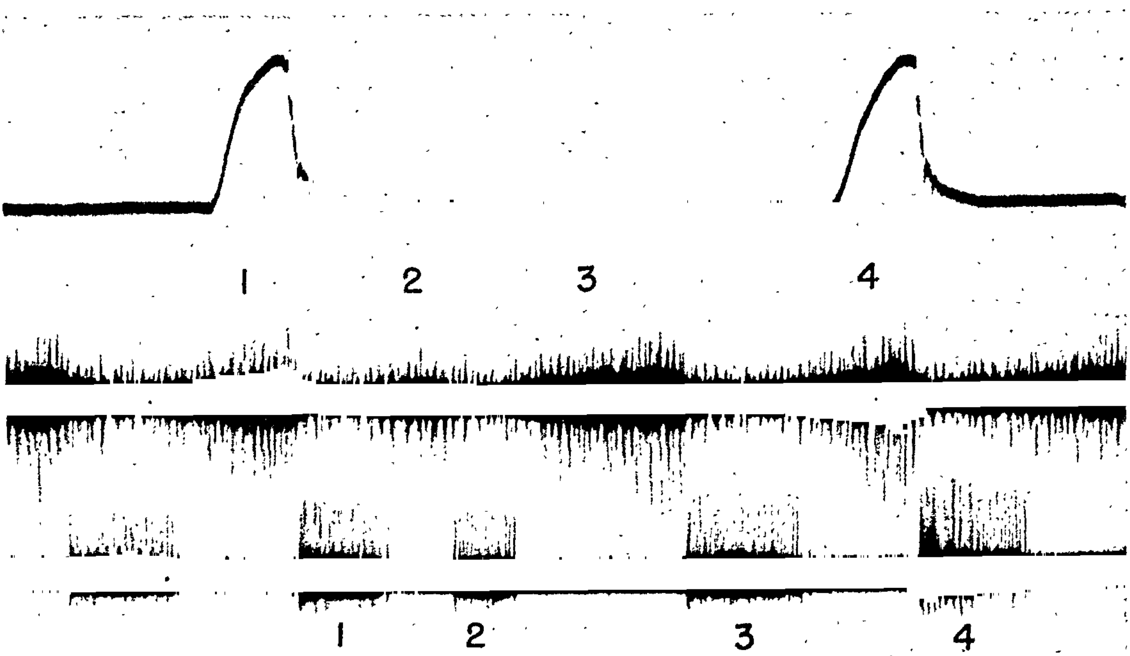

Fig. 2. Effects of intensity of discharge of the vagal stretch receptors on the contraction of the thyroarytenoid muscle. The upper record of tracheal pressure shows artificial inflation of the lungs during open pneumothorax. The upper electrical record shows fusillade contractions of the diaphragm. The lower record shows the activity of the thyroarytenoid muscle. During respiratory cycles 2 and 3 the lungs are collapsed and vagal expiratory drive is uniformly low. During cycles 1 and 4 the vagal expiratory drive is of the slowly waning type. For further discussion see text.

This had the advantage of bringing only every third inspiration (1 and 4 of the upper electrogram) into phase with each artificial inflation of the lungs and allowing two intervening respiratory cycles with the lungs permanently collapsed (2 and 3) to be used for controls. We are thus enabled to compare the activity of the expiratory center under an initially powerful but waning vagal drive (expirations 1 and 4) with the activity under a uniform or zero vagal drive (expirations 2 and 3). It will be seen at once that the discharge is of the steady state type in expirations 2 and 3 and of the rapidly augmenting and slowly waning type in expirations 1 and 4. Since this modification of expiratory contractions by artifical ventilation

of the lungs is usually completely abolished during double vagal block it is concluded that the vagal stretch reflex is probably the most important factor contributing to the slowly waning expiratory contraction during normal breathing. The results of figure 2 combined with figure 1 show furthermore that vagal activity is not only able to elicit a purely reflex contraction but can reflexly modify the steady state activity such as occurs during zero or uniformly small vagal drive. From this it follows that the rapidly augmenting and slowly waning expiratory contraction can be either a purely reflex activity or a modification of the steady state activity.

What then is the nature of the expiratory discharges and wherein do they differ from the slowly augmenting inspiratory discharge? Acceleration of frequency in the latter discharge (since it was obtained in the absence of all periodic afferent impulses) was tentatively attributed to the augmenting action of the recurrent collaterals (Gesell, Atkinson and Brown, 1940). Since acceleration is missing in the steady state discharge these structures have been omitted in the architectural schema of the expiratory half-center. Recruitment of new active units in the course of a slowly augmenting discharge has also been explained on a structural basis, but with the aid of recruiting collaterals. Since recruitment is absent in the course of a steady state contraction the recruiting collaterals have been omitted in the expiratory half center schema as well. While it is true that the steady state activity may vary with varying intensity of chemical stimulation, it still retains the steady state pattern. Such increased activity is regarded simply as an activation of a greater number of cells within the subliminal range. The relatively greater number of expiratory units active at the beginning of a rapidly augmenting and slowly waning contraction is accordingly attributed to an initially intense reflex activation of those cells lying in the subliminal fringe. This activation however diminishes as contraction progresses and vagal discharge weakens, thus producing the familiar phenomenon of decruitment.

The relatively greater incidence of the steady state expiratory contraction as compared with the rapidly augmenting and slowly waning contraction in routine electrical exploration of expiratory muscles is possibly related to the low degree of reflex sensitivity prevailing under ordinary experimental conditions. On the other hand it may be an indication of the relative unimportance of expiratory activity in the mechanics of eupneic breathing.

SUMMARY

The nature and origin of the expiratory activity patterns were investigated by recording the expiratory action potentials of the thyroarytenoid muscle of the dog during changing vagal activity controlled by artificial inflation of the lungs.

Intensity of contraction was found to vary with the degree of pulmonary

inflation. Since this effect was abolished by double vagal block it was concluded that the vagal stretch reflex is capable of driving expiratory muscles in a precise and machine-like manner.

When the lungs were inflated in a progressive manner the synchronous expiratory contraction was therefore of the slowly augmenting type. When however an initially high but slowly waning vagal discharge occurred in phase with the expiratory discharge a rapidly augmenting and slowly waning contraction was obtained. It was therefore concluded that the waning vagal activity occurring during normal expiration is an extremely important factor in the production of the rapidly augmenting and slowly waning expiratory contraction.

Architectural arrangements of the inspiratory and expiratory half-centers underlying the slowly augmenting and steady state contractions are discussed.

Since vagal activity was demonstrated to evoke a purely reflex activity as well as a reflex modification of a prevailing expiratory discharge it is concluded that the rapidly augmenting and slowly waning expiratory activity may be primarily a reflex response or a reflex modification of the steady state discharge.

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INFLUENCE OF RIGHT AND LEFT VENTRICLES ON THE ELECTROCARDIOGRAM[1]

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To explain the genesis of the normal electrocardiogram, earlier writers have postulated the summation of electrical events from the two ventricles, each ventricle acting as a unit, and giving rise to its own electrogram. These are designated as dextro- and levocardiograms, and are believed to be of opposite polarity, and slightly out of phase with one another (1). By blocking first one and then the other bundle-branch, following suggestions of Eppinger and Rothberger (2), Lewis obtained what he considered to be the initial portion of the dextro- and levocardiograms, and showed that their algebraic summation resulted in a curve that resembled the initial deflection of the normal electrocardiogram taken before the experimental procedures (3). Since this method did not extinguish activity in one or the other ventricle, but only postponed its occurrence, it was impossible to determine the nature of the entire dextrocardiogram or levocardiogram. Despite continued interest in the electrograms of the right and left ventricles (4), efforts to obtain complete curves, and to determine if they would summate to yield a normal T wave have hitherto been unsuccessful. There is, however, evidence that the character of the T wave in electrograms taken by direct leads "is determined by the algebraic summation of the monophasic action current recorded by each of the electrodes" (1, 5).

In these experiments we have obtained electrograms from the right and left ventricles by a method which reduces or extinguishes electrical activity at the surface of the opposite ventricle. This method has been described previously (6, 7) and is based upon the following two observations: *a.* The electrocardiogram is determined by activity at the surface of the heart. *b.* Potassium chloride in M/10 or M/5 solutions extinguishes electrical activity at the point of its application to the surface of the heart. By covering large portions of the surface of a single ventricle with filter paper

[1] This work was aided by a grant from the Fluid Research Funds, Yale University School of Medicine.

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soaked in M/5 KCl solution, it was thus possible to expose the electrogram of the untreated ventricle.

METHOD. Seven dogs, 12 cats, and 6 monkeys (5 rhesus and 1 mangabey) were employed in these studies. Animals were deeply anesthetized with sodium amytal and the heart exposed as described previously (6). Care was taken to preserve as much as possible of the over-lying soft tissue, and this was clipped together during recording to restore normal continuity as far as possible and to permit adequate conduction from this area. For the same reason the lungs were fully inflated during recording. A small slit only was made in the pericardium, so that this structure remained intact and served to hold the KCl pledget in place. Electrocardiograms were taken from the three conventional leads with the animal placed on its side, since it was found that the most striking changes were obtained with the animal in this position (8). Fifth molar potassium chloride solution was employed throughout these experiments.

The major difficulty encountered was caused by spread of solution from the area being studied. To avoid this it was necessary to remove all excess fluid from the pericardium, and from the chest cavity after each washing, and to use relatively dry pledgets. With these precautions spread of solution was minimized, and it was also found that the pledget itself showed less tendency to change its position. After each treatment, the pledget was removed, the heart and pericardium were thoroughly washed with warm Ringer-Locke solution, and time was allowed to permit the electrocardiogram to return to normal (6).

RESULTS. When a large portion of the surface of a single ventricle was treated, an electrocardiogram was obtained which resembled closely the monophasic records that have been derived directly from the surface of the heart (5, 8, 9). The record obtained by extensive damage to the left ventricle was upright, and began with a short downward Q wave, and its rapid upward limb was the R wave. Since it was produced by damage to the left ventricle, and since it preceded the opposite wave, it was interpreted as the record of preponderant electrical activity in the right ventricle, or the dextrocardiogram (fig. 1B). The monophasic-like record obtained by treatment of most of the right ventricle with potassium chloride was directed downward, and its rapid descending limb was the S wave. It was interpreted as a record of the electrical activity of the left ventricle, or the levocardiogram (fig. 1C).

When these curves were plotted using the Q wave as a point of reference, and the major deflections summed algebraically, a complex was obtained which closely resembled the normal electrocardiogram (fig. 2). In experiments in which an inverted T was present it was observed that the durations of the dextro- and levocardiograms, measured between the major deflections at the isoelectric level, were equal. Consequently the levocardiogram persisted for some time after the termination of the dextrocardiogram, since it commenced somewhat later. In a single experiment in which an upright T wave was observed, the levocardiogram was of shorter duration than the dextrocardiogram, so that even though it began later, it was completed

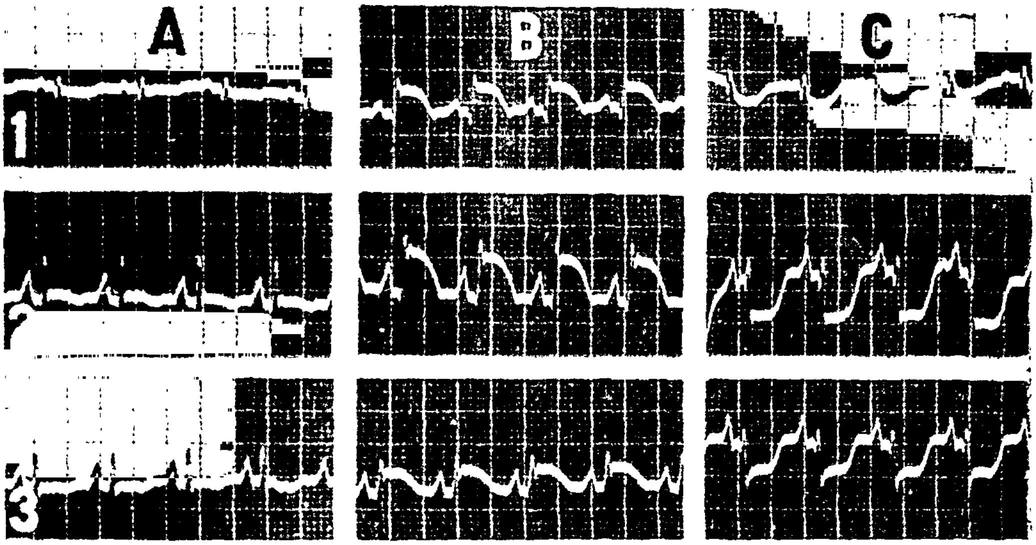

Fig. 1. Dog. November 9. A. Control, leads 1, 2 and 3. B. Dextrocardiograms in the three leads. C. Levocardiograms in the three leads. The levocardiogram is imperfect in lead I.

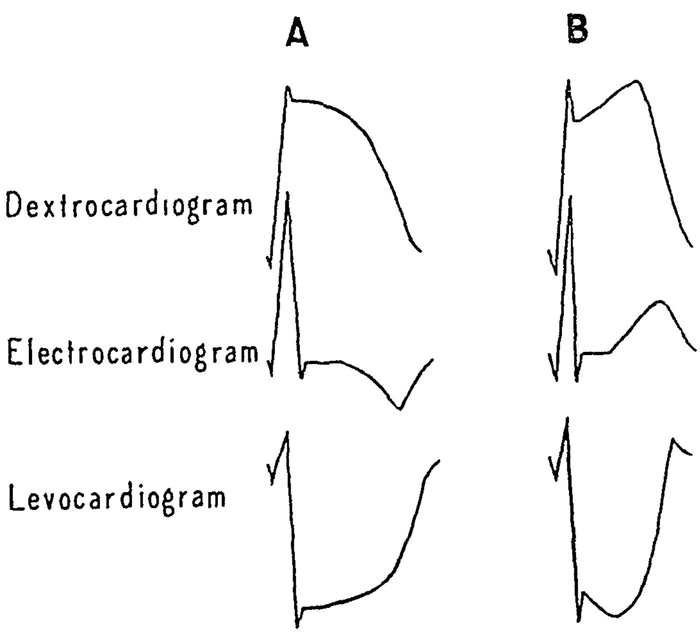

Fig. 2. A diagram illustrating the factors regulating the direction of the T wave. In A the dextro- and levocardiogram are of equal duration, but the levocardiogram, starting later, ends later. A negative T wave would result as shown in the center figure, which represents the actual summation. In B the levocardiogram begins later, but is of shorter duration, so that it ends earlier than the dextrocardiogram. This would form an upright T wave.

before the termination of the dextrocardiogram (fig. 3). This relative shortness of the levocardiogram was accentuated when the animal was cooled (fig. 3).

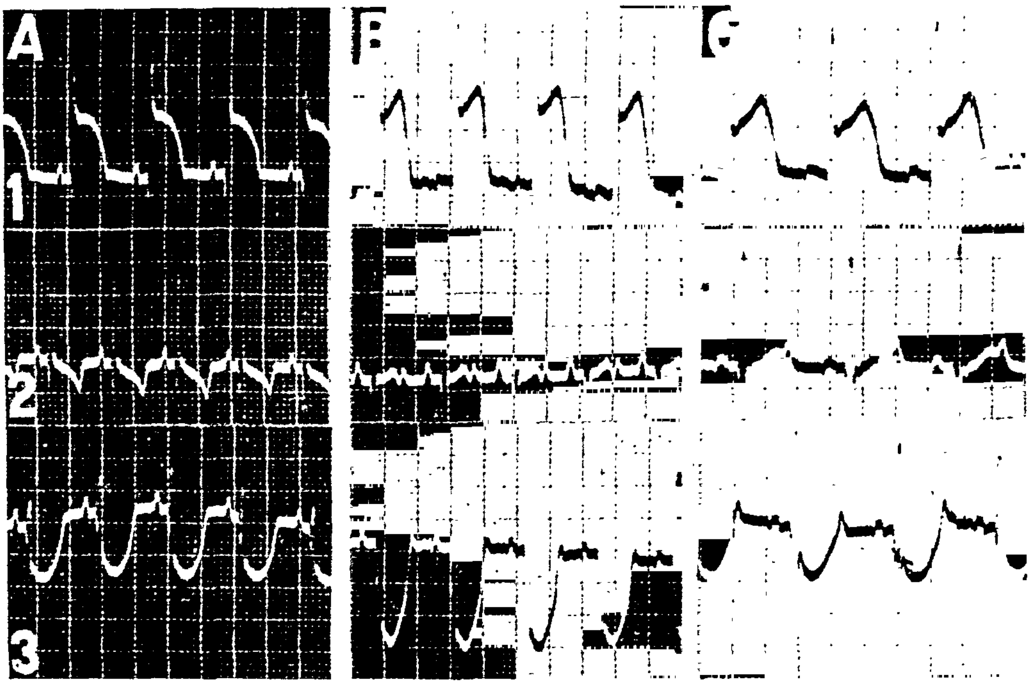

Fig. 3. In A are shown the above dextrocardiogram and below the levocardiograms from lead II of a typical experiment in which the T wave was inverted. The duration of the two electrograms is the same. In B are shown the dextro- and levocardiograms from an experiment with an upright T. The duration of the levocardiogram is such that despite the later onset, it ends 0.02 to 0.03 sec. before termination of dextrocardiogram. There is also a distinct difference in contour. Both factors are accentuated in C, taken with the animal cooled to 30°C. Number 2 in each group shows the control electrocardiogram, also from lead II.

Discussion. In the experiments reported here we have succeeded in recording what appear to be the electrograms of individual ventricles. This was accomplished by extinguishing the electrical activity of one ventricle by covering its surface with M/5 potassium chloride, which permitted registration of the contribution made to the electrocardiogram by the opposite ventricle. The effects of potassium chloride were rapidly reversible, permitting the recording of both the dextro- and the levocardiogram from the same heart.

A striking similarity was found between the electrogram of an entire ventricle derived from the conventional leads and the monophasic action current obtained from a single point on the surface of the heart. There were, however, some variations between the dextro- and levocardiograms and pure monophasic curves. The dextrocardiogram always showed a Q

wave whenever this was present in the electrocardiogram. The levocardiogram showed a Q wave and a small R wave. Inasmuch as technical reasons make it impossible completely to cover the ventricle with the solution, it is possible that the R wave in the levocardiogram was a remnant of right ventricular activity not abolished by potassium chloride. The more complete the coverage, however, the less prominent the R wave became.

The dextro- and levocardiograms usually showed a sharp spike before the plateau of the wave. This spike is frequently seen also in monophasic action currents. It tended to be reduced as the block of the ventricle by potassium chloride became more complete, and may therefore not represent a characteristic of the curve from the opposite ventricle. It was, however, seen so frequently that it cannot yet be ruled out as a part of the complex.

In all conventional leads the dextrocardiogram was upright, and the levocardiogram was inverted. The dextrocardiogram preceded the levocardiogram by a short interval, and its ascending limb was the R wave. The apex of the dextrocardiogram thus coincided with R, and the apex of the levocardiogram with S. In the dog the levocardiogram therefore began approximately 20 msec. after the onset of the dextrocardiogram.

Some conclusions may now be drawn concerning the genesis of the electrocardiogram. The monophasic character of the electrogram of each ventricle indicates that each ventricle fires as a unit with practically simultaneous discharge of all its fibres. The right ventricle is activated first, and contributes Q and the upstroke of R. At this time the left ventricle is activated, and the potential is equalized, forming the downstroke of R and S. During the ensuing S-T interval both ventricles are active, but their opposite electrical potentials neutralize each other at the distant leads. If cessation of activity in the right ventricle occurs before that of the left, a downward T wave results. This is the usual finding in the dog in these experiments (figs. 2 and 3). Occasionally, the dextrocardiogram persists after the levocardiogram has ended, even though it began earlier. In such cases, the T wave is upright, because there is now a residue of the upright dextrocardiogram (figs. 2 and 3).

The presence of a Q wave preceding both the dextro- and levocardiogram suggests that it may arise in tissue activated before the surface of the two ventricles.

SUMMARY

1. Extinction of electrical activity of the left ventricle by covering its surface with M/5 KCl permits the recording of the electrogram of the right ventricle, which may be designated as the dextrocardiogram. It is a monophasic-like curve which arises from the R wave, and is upright in the three conventional leads.

2. The levocardiogram is similarly obtained by blocking the electrogram of the right ventricle. It is a monophasic-like wave which arises with the S wave, and thus is somewhat later in time than the dextrocardiogram. In all conventional leads it is inverted.

3. The electrocardiogram represents the algebraic sum of the dextro- and levocardiograms.

4. Similar results were obtained from dogs, cats and monkeys.

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THE SIGNIFICANCE OF DISPLACEMENT OF THE RS-T SEGMENT[1]

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Certain specific electrocardiographic patterns have come to be associated with the acute and later phases of coronary occlusion (1). In the early phases these consist of elevations or depressions of the RS-T segment, often with elevation in one lead and depression in another. Elevation in lead I and depression in lead III are considered to be due to infarction of the anterior portion of the left ventricle, while depression in lead I and elevation in lead III are interpreted as results of infarction of the posterior wall of the left ventricle (1). The significance of such patterns has been derived chiefly from clinico-pathological correlations, and the reason for these changes remains in doubt.

Experiments reported in a preceding paper indicate that the normal electrocardiogram results from interference between electrograms of the two ventricles, that of the right ventricle preceding by a short interval the electrogram from the left ventricle (2). These electrograms resembled greatly monophasic action currents recorded from the mammalian heart, and in the three conventional leads the dextrocardiogram was upright, while the levocardiogram was inverted.

These electrograms were produced by reducing or abolishing the electrical activity of a single ventricle by covering a large part of its surface with pledgets of filter paper soaked in M/5 KCl. When smaller squares of filter paper were employed less pronounced results were obtained, but they always consisted of elevations of the R-T interval indicating left ventricular damage or depression of the S-T segment indicating damage to the right ventricle. This paper reports experiments which throw further light on the significance of deviations of the RS-T interval, particularly when elevation is found in one lead and depression in another.

METHODS. The experiments were carried out on the animals employed in the previous series (2), using the same method of application of squares of filter paper

[1] This work was aided by a grant from the Fluid Research Funds, Yale University School of Medicine.

soaked in M/5 KCl, except that the pledgets were small enough to cover only a small portion of the surface of the heart. In some experiments the area treated was confined to a single ventricle, while in others contiguous areas of both ventricles were treated. Records were taken with the animal in various positions to determine the influence of this factor on the magnitude of changes.

RESULTS. A. *Single ventricular effects.* When the pledget was placed only upon the left ventricle the alterations observed consisted always of an upward displacement of the RS-T segment, while damage to the right ventricular surface showed invariably a downward displacement (figs. 1–2). The direction of these changes was the same whatever portion of the left or right ventricle was studied, but the magnitude depended upon 1, the size of the area affected; 2, its position on the heart, and 3, the position of the animal. Changes so produced were always maximal in lead II.

The first factor has been emphasized in a previous communication (3), namely, that the extent of the displacement is proportional to the area affected. The other two points may now be reported more fully. The lateral and anterior surfaces of each ventricle were relatively inactive with the animal on its back (fig. 2, D), while the apex and posterior surfaces yielded maximal changes. When the animal was placed on its right or left side, however, treatment of the anterior and lateral surfaces produced changes equalling those from the apex or posterior surfaces (fig. 2, E, F). In monkeys in which the left lung was collapsed, while respiration was maintained by the unaffected right lung, minimal changes only were obtained from the anterior surface of the left ventricle. When the lung was inflated, more striking changes were recorded. Changing the position of the animal from the back to the sides occasionally reduced the extent of RS-T displacement when the pledgets were placed on the posterior surface of the heart. Great care must be taken to insure that the potassium chloride solution does not spread beyond the area treated. This readily happens if excess fluid collects in the pericardial cavity and the position of the animal is altered. In this event both ventricles may be involved and the results will be complicated.

B. *Combined right and left ventricular effects.* When the pledget was placed over the septum so that it covered parts of both ventricles, mixed effects were obtained. Placed anteriorly, it produced in lead I an upward displacement of the RS-T segment, and a downward one in lead III (fig. 3). The downward displacement in lead III was usually greater than the upward deviation in lead I, and as a result, the S-T segment in lead II was usually also depressed.

Combined effects from the apex and whole posterior septal region showed the opposite picture; the RS-T segment in lead I was invariably deflected downward, while in III it was upward. In lead II it was usually elevated (fig. 4). This seemed to be determined largely by the relative areas involved on each ventricle (fig. 4).

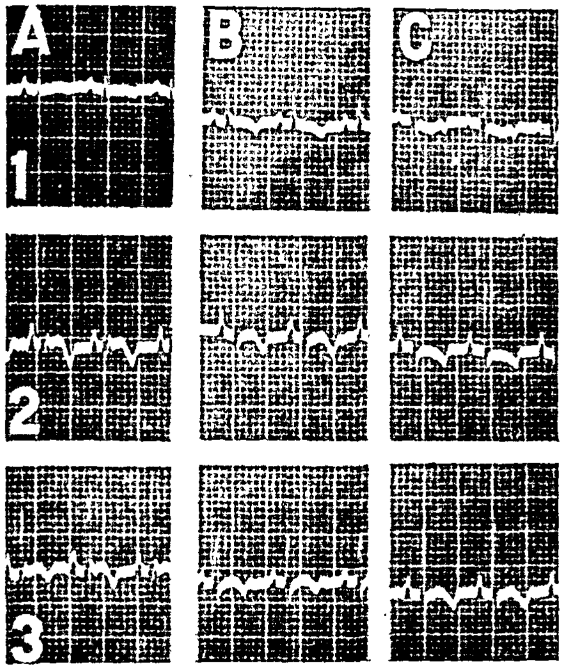

Fig. 1. Dog. November 24; 4.3 kgm. Control in leads I, II, III, with animal on back (A), on left side (B), and on right side (C). These serve as control for figure 2.

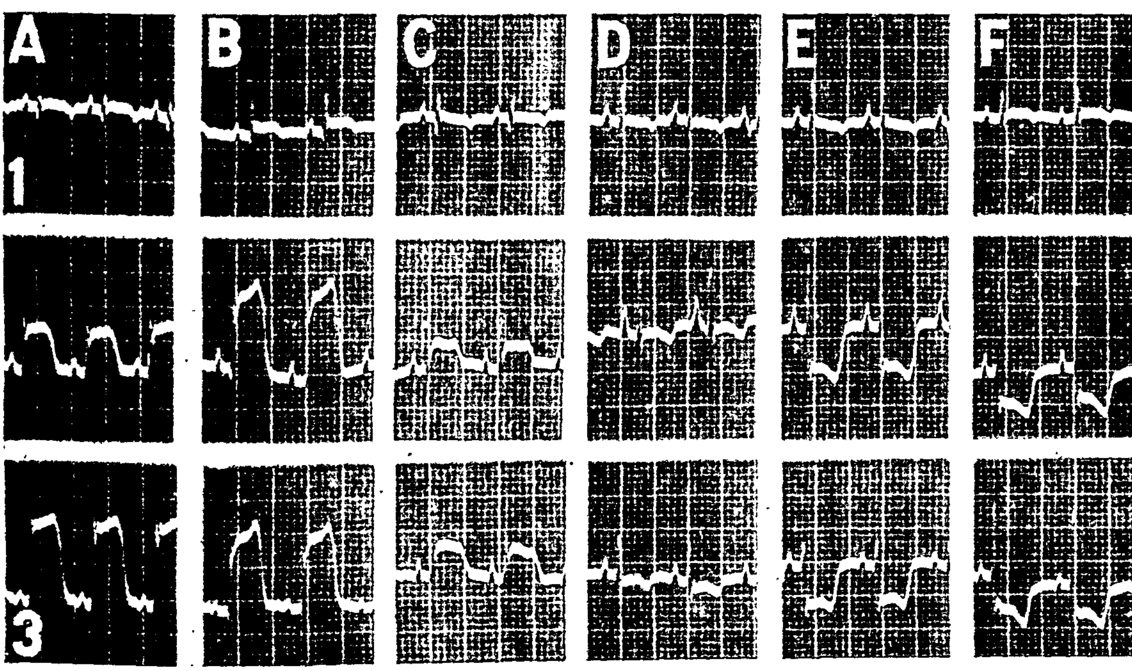

Fig. 2. Same animal as in figure 1. In A, B and C, pledget 2.5 x 1.5 cm. on anterior-lateral surface of left ventricle. Leads I, II, III. Back (A), left (B), right (C). In D, E, and F, the pledget was on the anterior lateral surface of the right ventricle. Animal on back (D), left side (E), right (F). This figure illustrates the elevation produced by purely left ventricular lesions and the depression following purely right ventricular lesions. These changes were most marked when the animal was on its left side, and are most prominent in lead II.

Shift in the position of the animal in these experiments produced marked changes in the magnitude of the effects evoked from the anterior surface (fig. 3, D, E, F), while alterations of position did not influence results from the posterior surface as greatly. Change in the position of the animal did not influence the direction of the displacement, but only its extent, provided that spread of solution was avoided.

In the monkey the left ventricle seemed to be preponderant at the apex and posterior surface, so that frequently a pledget placed at the

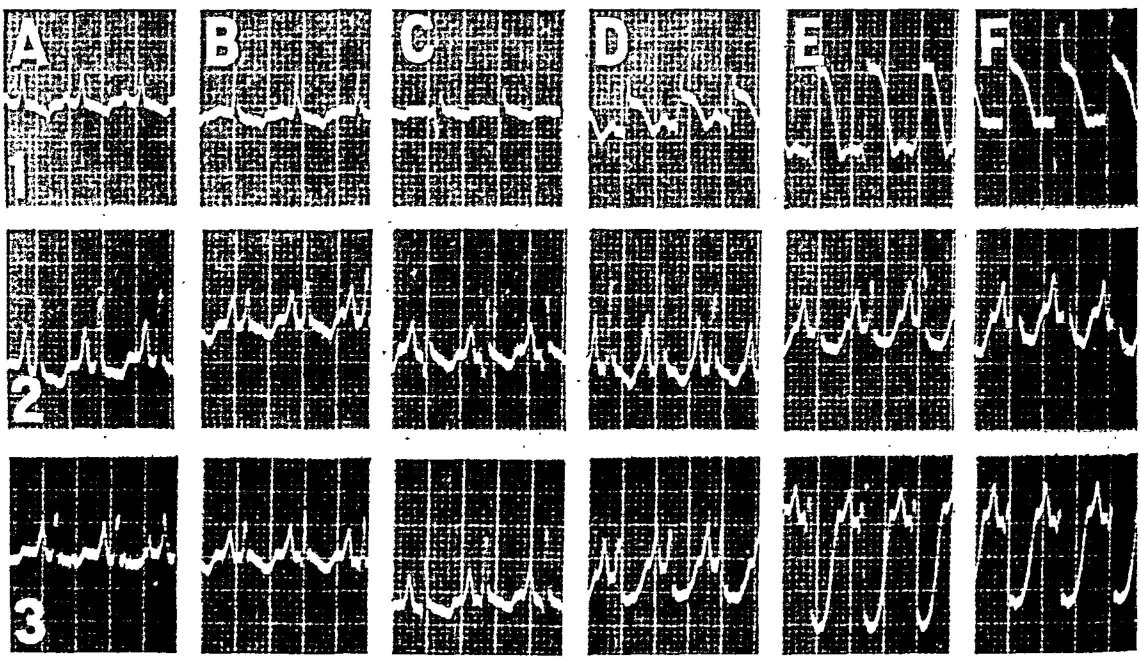

Fig. 3. Dog. January 5; 7.2 kgm. A, B, C, control from back (A), left (B), and right side (C) in leads I, II, and III. Pledget 3.0 x 3.0 cm. over anterior septum at base. Back (D), left (E), right (F). This figure illustrates strikingly the elevation of R-T in lead I and the depression of S-T in lead III produced by an anterior lesion involving adjacent surfaces of both ventricles. Maximal changes were recorded with the animal on its left side. The interference of the dextrocardiogram in lead I and the levocardiogram in lead III produced an almost normal T wave in lead II.

septum produced only left ventricular effects. This may be accounted for by the fact that in the monkey the bulk of the posterior surface of the heart is made up of the left ventricle. In the dog the right ventricle has a larger representation in the posterior surface, and in this animal mixed effects were always obtained.

C. *Changes in Q R S.* Upward deviation of the R-T segment produced by injury of the left ventricle invariably occurred somewhere on the downstroke between R and S. With larger elevations the amplitude of R increased, forming a wave having close resemblance to a monophasic

action current (fig. 2, A, B). When depressed S-T intervals were obtained from right ventricular damage, the take-off occurred somewhat later, after the S wave was completed. In these records, the R wave diminished as the S wave increased until in some records monophasic-like waves were found which were opposite to those obtained from the left ventricle, and occurred a little later in the cycle (fig. 2, E).

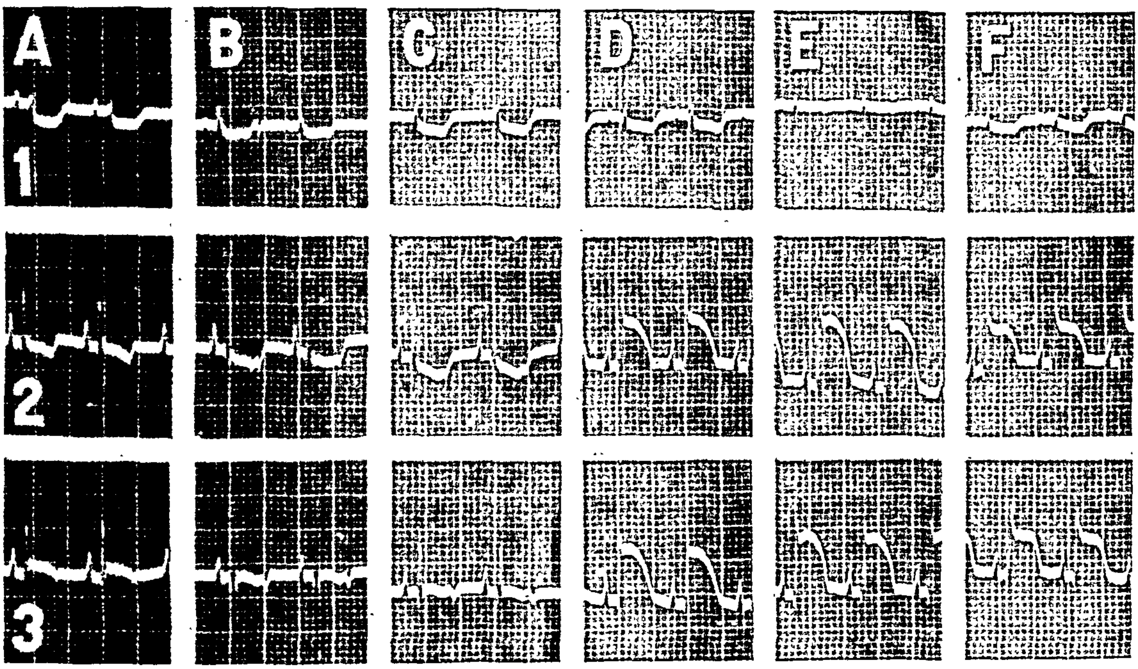

Fig. 4. Dog. November 2; 5.0 kgm. Pledget 1.5 x 1.5 cm. Pledget placed across septum on posterior surface. In A, B, and C it was near the base, and almost entirely on the right ventricle, while in D, E, and F it was nearer the apex and largely on the left ventricle. This figure shows how the magnitude of changes in the various leads is influenced by the relative areas involved. In A, B, and C the pledget was largely on the right ventricle; and both leads I and II show the depression of the S-T segment characteristic of a right ventricular lesion. The elevation of R-T indicative of left ventricular involvement is seen only in lead III when the animal was on its back (A 3). In D, E, and F the pledget was mostly over the left ventricle, and now marked elevation is seen in leads II and III.

Discussion. A. *Significance of RS-T displacement.* These experiments give a definite significance to displacements of the RS-T interval. Elevation of R-T in any lead denotes damage to the surface of the left ventricle, while depression of S-T indicates damage to the right ventricle. The mechanism of these deviations is indicated by the nature of the action of potassium chloride (4). When electrical activity is extinguished in a portion of the heart by potassium chloride, an imbalance is created between the dextro- and levocardiograms, and the undamaged ventricle preponderates, producing either an elevation or a depression of the RS-T segment,

depending upon which ventricle is injured. As more and more of the ventricle is damaged, the opposite ventricle becomes more and more preponderant, until finally pure dextro- or levocardiograms remain (2).

B. *Localization of injury.* When only one ventricle was involved the displacement was similar in all leads regardless of the region of that ventricle affected, and was always maximal in lead II. It was possible to determine whether these lesions were anterior or posterior by the influence of alteration in position of the animal on the magnitude of changes recorded. Electrocardiographic evidence of anterior lesions in either ventricle was always magnified by placing the animal on its side. On the other hand, when the lesion was on the posterior surface of either ventricle, maximal changes were observed with the animal on its back.

More exact localization was possible when adjacent areas of both ventricles were involved. Then lead I became more active than was seen following lesions of single ventricles, and was always opposite to lead III, while lead II showed the least changes. Elevation of RS-T in lead I and depression in lead III characterized anterior lesions, while depression in lead I and elevation in lead III denoted posterior lesions. Lesions at the apex produced changes similar to those of posterior lesions.

The observation that a pledget over the septum involving contiguous areas of both ventricles showed right ventricular effects in one lead and left ventricular in another, indicates that action currents from contiguous areas are not algebraically summated in leads I and III. This will be considered in a subsequent communication.

The electrocardiographic changes produced by pledgets covering part of both ventricles compare closely with those ascribed to acute infarction. Barnes describes the characteristic changes in the standard leads following anterior lesions as an elevation of the R-T segment in lead I and a depression of the S-T segment in lead III (1). When these effects are marked, they have the appearance of monophasic waves. Posterior lesions evoke downward deviation of the S-T segment in lead I, and upward deviation in lead III.

In the experiments reported here, such patterns have been found only as the result of injury to contiguous areas of both right and left ventricles.

C. *Influence of position of animal.* We interpret the influence of change in position as due to alteration in conductivity from the involved areas. The reason that the apex and posterior surface consistently yielded maximal changes with the animal on its back is probably due to the contact of these regions with good conductors, as Lindner and Katz have pointed out (5). Anteriorly, there is little contact with surrounding tissues when the animal is on its back, but when the animal lies on its side, the anterior surface of the heart falls against the root of the lungs and the anterior chest wall, and thus increases its contact with conducting tissues. In this

position, there is a great increase in the effect of anterior applications. This emphasizes further the necessity for adequate conduction from the surface of the heart to reveal the effects of damage, and suggests the use of shift of position as a means of localizing the site of injury. This procedure is the only means of distinguishing anterior from posterior lesions when damage is restricted to a single ventricle, since in these circumstances all leads record either elevation or depression, depending on the ventricle involved.

SUMMARY

1. Elevation of the R-T segment of the electrocardiogram in the dog, cat, and monkey indicates injury to the left ventricle.

2. Depression of S-T indicates injury to the right ventricle.

3. When the injury is restricted to a single ventricle, the RS-T interval in all three conventional leads is deflected in the same direction.

4. Elevation of R-T in one lead, and depression of S-T in another indicate that the damage involves contiguous areas of both ventricles.

5. Elevation in lead I and depression in lead III indicate an anterior lesion.

6. Depression in lead I and elevation in lead III indicate an apical or posterior lesion.

7. Lesions of the anterior surface of the heart are recorded best with the animal on its side, while lesions of the posterior surface of the heart are recorded best with the animal on its back.

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THE FACTORS DETERMINING THE DIRECTION OF THE T WAVE: THE EFFECT OF HEAT AND COLD UPON THE DEXTRO- AND LEVOCARDIOGRAM[1]

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A satisfactory explanation of the formation of the T wave of the mammalian electrocardiogram is still required. Much work on the subject of the T wave was done by earlier investigators of the electrophysiology of the heart but their studies were carried out on the amphibian heart, and have never been adequately extended to the mammal. In 1880 Burdon-Sanderson and Page (1) first proposed the interference theory to explain the terminal portion of the electrocardiogram of the tortoise. They studied the electrogram obtained by direct leads at the base and apex, and concluded that the initial deflection or "spike" was formed of two elements. The rising phase was considered due to the onset of electronegativity at the base, and its rapid descending phase was supposed to result from the activation at the second electrode at the apex which equalized the potential between the two electrodes, although both regions remained negative to non-active regions. The following isopotential phase they explained as resulting from the interference of the electrical activity at both points. The electrical activity of the heart then ceased in the order in which it began, and therefore negativity at the base subsided while that at the apex continued, producing an inverted end-deflection, or T wave.

The hypothesis was therefore first applied to the interference between apex and base. It was later altered, to apply to interference between the right and left ventricles. Lewis compressed alternately the right and left bundle branches in an attempt to obtain at least the initial portion of the electrogram from each ventricle. Algebraical summation of the initial portions of these complexes did in fact yield a complex comparable to the normal QRS (2).

Concerning the final portion of the electrocardiogram, Lewis wrote: "T can be regarded also as the product of the end-deflections of right and

[1] This work was aided by a grant from the Fluid Research Fund, Yale University School of Medicine.

left ventricle. Unhappily, we have no certain means of ascertaining either the direction or value of the end-deflections of the true dextrocardiogram and levogram. I have in mind the real end-deflections and not those which appear when the left and right bundle branches are divided; for these, as I have explained, are not reliable indications. Experiments in which one or the other ventricle is removed and the resultant curve observed are perhaps too crude to possess material value from this point of view."

In a former communication there has been described a method for obtaining the dextro- and levocardiograms (3). It consisted of temporarily reducing or extinguishing the electrical activity of a single ventricle by the application to its surface of M/5 KCl, so that the unopposed activity of the other ventricle was recorded.

It was seen that complexes thus recorded resembled the monophasic action currents obtained from the surface of the mammalian ventricle, and in all conventional leads, the dextrocardiogram was directed upward, while the levocardiogram was directed downward (fig. 4B). The dextrocardiogram began earlier in the cardiac cycle as a continuation of the R wave, while the levocardiogram was a continuation of the S wave (fig. 4F).

When the duration of each component was the same, the levocardiogram, beginning later, ended later than the dextrocardiogram. Algebraic summation of such complexes would be expected to produce an inverted T wave, and in fact an inverted T wave was found in the normal electrocardiogram. This is illustrated in figure 1A.

In the following experiments further studies of the factors determining the direction of the T wave have been carried out. If the hypothesis be true that the T wave is created by the interference of dextro- and levocardiograms, then alterations in the duration of each component should change materially the direction and contour of the T wave. If the duration of the dextrocardiogram or upward component were shortened, the T wave should become more negative due to the increased amount of unopposed levocardiogram. The duration of the Q-T interval should not be altered. This situation is illustrated in figure 1B. If on the other hand the dextrocardiogram were lengthened, there should then be a residue of unopposed dextrocardiogram, and a positive T wave should occur, as in figure 1C. Here the duration of Q-T should be prolonged. The same considerations apply to changes in the levocardiogram, with the dextrocardiogram remaining normal. Here opposite effects would be expected as illustrated in figure 1D and E. Shortening the levocardiogram should produce an upward T, without changing the duration of the complex, while lengthening the levocardiogram should result in an inverted T of longer duration.

It is known that heating and cooling the animal as a whole shortens and

lengthens the ventricular complex of the electrocardiogram. In these experiments heat and cold were applied separately to each ventricle in an attempt to shorten or lengthen single components of the electrocardiogram, and to determine whether the results thus obtained would confirm the theoretical predictions made above.

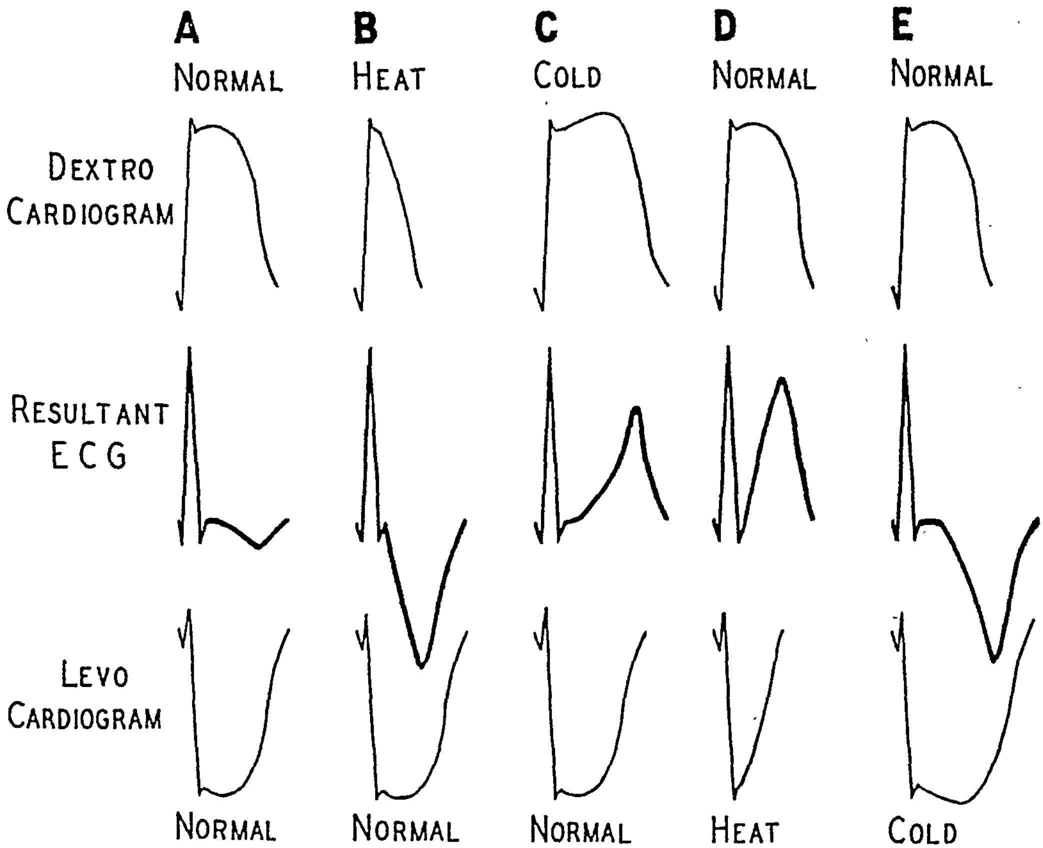

Fig. 1. This figure illustrates the theoretical basis for the experiments reported here. In A are shown at the top a normal dextrocardiogram, and below a normal levocardiogram. Their relative proportions are taken from previous experiments. The dextrocardiogram shows a Q, while both a Q and a small R are shown in the levocardiogram. In the middle is shown the result of an algebraical summation of the dextro- and levocardiograms. Since the duration of the two components is the same, measured from the major deflection at the isoelectric line, the dextrocardiogram terminates before the levocardiogram, and a negative T results. In B and C are shown the results on the T wave of shortening and lengthening the dextrocardiogram, while the levocardiogram remains normal. In D and E the dextrocardiogram remains normal while the levocardiogram is shortened and lengthened. When either the dextro- or levocardiogram is prolonged, a prolonged Q-T interval results.

Methods. Seven dogs were employed. They were anesthetized with sodium amytal, and the heart exposed under artificial respiration, through an incision over the fifth left rib. Electrocardiograms were obtained from the three conventional leads with the animal on its right side, with the lungs fully inflated and the skin wound closed with clips.

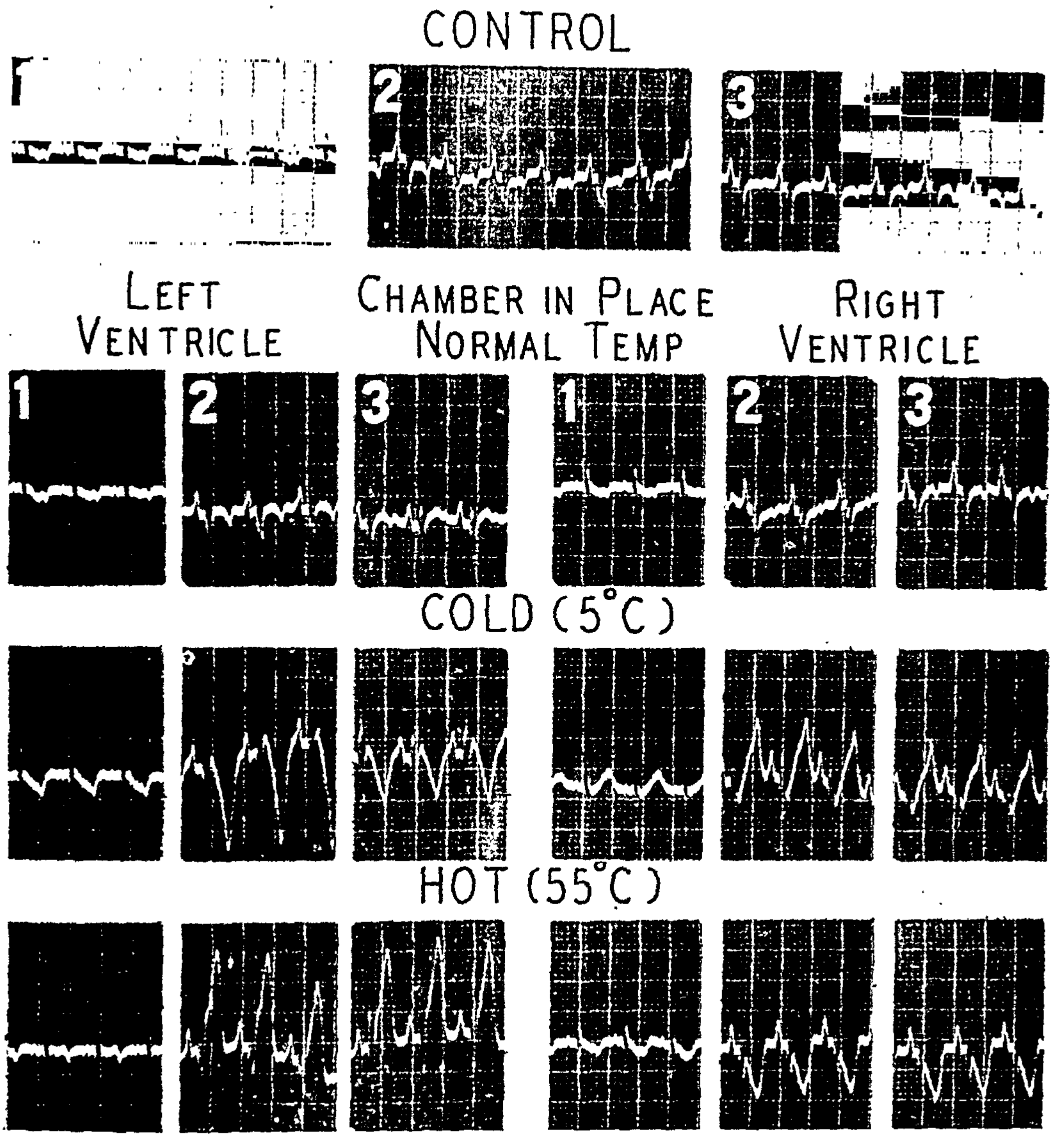

Fig. 2. April 29, 1940; 9.5 kgm. male dog. At the top are control records from leads 1, 2 and 3, taken after all operative procedures. All records were taken with the animal on its right side, with the lungs fully inflated, and the skin wound closed by clips. Next below are controls with the chamber in place on each ventricle, but at body temperature (35°–36°C). Cooling the left ventricle produces a negative T while cooling the right ventricle produces an upright T. As can be seen readily from the relation to the P wave, these complexes are longer than normal. Finally are shown the effects of heating the left ventricle and the right ventricle. Here it is seen that the whole complex is of normal duration.

Areas of each ventricle were cooled or warmed by means of a thin hollow chamber of pure tin curved to fit the surface of the heart. Through this chamber was circulated water from reservoirs at about 55°C. and 5°C. The diameter of the disk was 3.0 cm., and its thickness 3.0 mm. Dextro- and levocardiograms were obtained by applying to the surface of the heart squares of filter paper soaked in M/5 KCl.

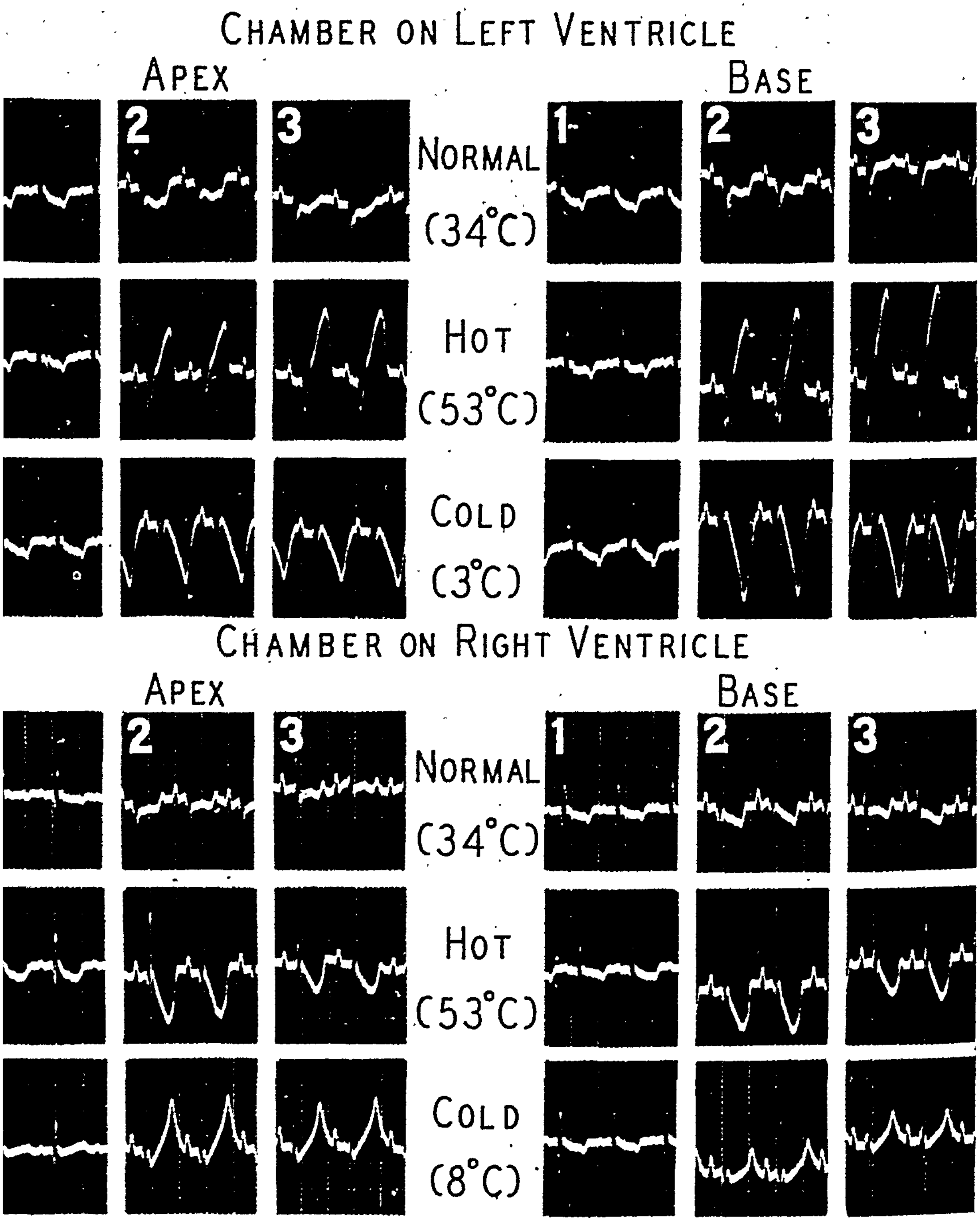

Fig. 3. June 14, 1940; 10.0 kgm. male dog. The effect of heating and cooling the base and apex of each ventricle is shown. The character of the alterations in the T wave is the The only differences are slight variations in the magnitude of the effects.

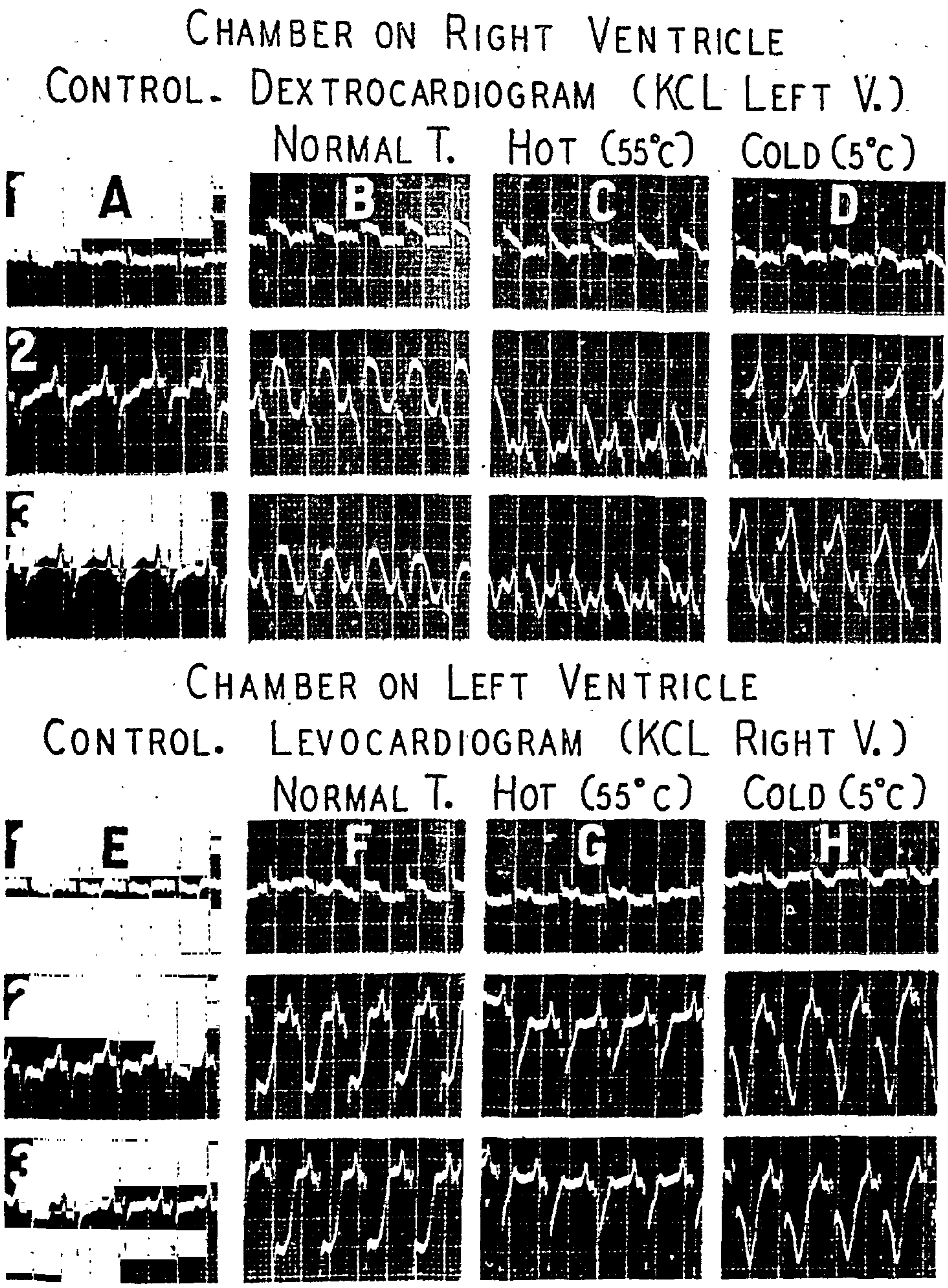

Fig. 4. Same experiment as figure 2. A. Control from three leads after all operative procedures, with chamber in place on the right ventricle at normal temperature. B. KCl pledgets were then placed over much of the left ventricle, giving a normal dextrocardiogram. In C the right ventricle was heated while the pledgets remained in place on the left ventricle. Records show shortening of the complex and disappearance of the plateau. In D the right ventricle was cooled. The dextrocardiogram was lengthened, and the plateau exaggerated. In E, F, G and H, the same series of experiments was carried out on opposite ventricles. The right ventricle was blocked with KCl to reveal the normal levocardiogram (F) which was affected by heat (G) and cold (H) exactly as was the dextrocardiogram.

RESULTS. Results in all experiments were uniform. They were as follows:

a. Controls with the disk in place on each ventricle at body temperature, showed no appreciable changes in the electrocardiogram.

b. Cooling the surface of the left ventricle invariably produced a sharply inverted V-shaped T wave, with prolongation of the duration of Q-T

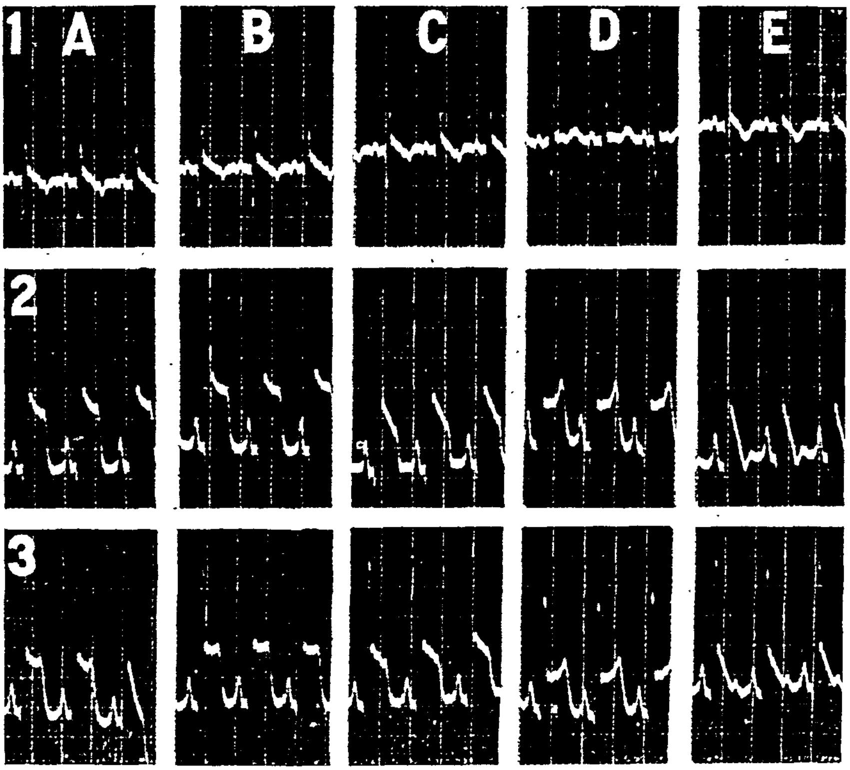

Fig. 5. June 8, 1940; 18.5 kgm. male dog. A. Dextrocardiogram produced by blocking left ventricles with KCl. The dextrocardiogram is best shown in leads 2 and 3. B. The region of the heart covered by the pledget was warmed by circulating water at 55°C through a chamber placed over the KCl pledget, which was of very thin filter paper. C. The region treated by KCl was cooled by circulating water at 5°C through the chamber. In neither B or C is there any alteration in the duration of the complex. In C there is a reduction in amplitude that is explained by the gradual decay of the potassium effect. In D and E the opposite ventricle (right) was cooled and heated respectively, producing the characteristic effect on the duration and contour of the complex. The same experiments carried out on the levocardiogram yielded identical results.

(fig. 2). Cooling the right ventricle produced an upright T wave which was also prolonged (fig. 2).

c. Heating the surface of the left ventricle produced an acutely upright T wave of normal duration, while heating the surface of the right ventricle produced a sharply inverted T wave of normal duration (fig. 2).

d. All areas of a given ventricle yielded qualitatively similar effects

when heated or cooled. Apical and basal areas acted similarly (fig. 3). This was confirmed further in two experiments where a smaller chamber was employed so that it was possible to investigate four separate areas on left ventricle, and three on the right ventricle. The only differences were in the magnitude of changes produced, and in the lead in which changes from the various areas were best recorded.

The nature of the dextro- and levocardiograms with heat and cold. To determine whether heating and cooling the surface of a single ventricle actually modifies the duration of the dextro- or levocardiogram as postulated, the separate ventricles were heated and cooled, while the opposite ventricle was blocked with KCl. Figure 4 summarizes the results, which were uniform in all experiments, and shows clearly that both the dextro- and levocardiograms were shortened materially by heat, and considerably lengthened by cold. In addition, changes in the contour of the complexes were produced.

As a control, the ventricle blocked by KCl was also heated and cooled, and this procedure did not alter the duration of the electrogram (fig. 5). These experiments confirm the hypothesis that the electrograms recorded after application of KCl to a single ventricle are the electrograms of the opposite untreated ventricle.

Discussion. The point of view which led to these experiments was first expressed by Burdon-Sanderson and Page, who showed clearly that heating the base of the tortoise heart caused an increased inversion of T. Since then several workers have employed the method, but continued the hypothesis that interference occurs between apex and base (Bayliss and Starling, 4; Mines, 5; Smith, 6). In 1909 Eppinger and Rotherberger froze the surfaces of the right and left ventricles with a spray of ethyl chloride, and noticed that in the first instance an upright T wave was produced, while in the second the T wave was inverted (7). In a previous paper from this laboratory it was shown that heat applied to the left ventricle at the apex produced an upright T wave, while cold caused an inversion (8). In the present experiments these observations are extended to both ventricles and an explanation of the T wave changes produced by heat and cold is given, based upon the influence of temperature upon the dextro- and levocardiograms.

It was predicted that since the T wave is produced by the interference of the terminal portions of the electrograms from right and left ventricles, modifications should occur in the T wave whenever the duration of these components is altered. In these experiments a single component was altered by applying heat and cold to the surface of a single ventricle. Heat actually shortened, and cold lengthened the dextro- or levocardiogram. When the dextrocardiogram was lengthened by cold, an upright prolonged T wave was obtained, and when the dextrocardiogram was

shortened by heat, a downward T wave was found, of normal duration. Lengthening the levocardiogram by cold produced a prolonged inverted T wave, and shortening the levocardiogram by heat gave rise to an upright T wave of normal duration. These changes were exactly as predicted theoretically.

These experiments afford additional proof that the interference which results in the normal electrocardiogram takes place between the two ventricles acting as units, and not between apex and base. In previous experiments (3) it was shown that application of small squares of filter paper soaked in M/5 KCl to various parts of the surface of a single ventricle always resulted in the displacement of the S-T segment in a single direction. In conformity with these results it is now seen that changing the temperature of various regions of the same ventricle always affected the direction of the T wave in the same manner.

It was clear from this study that the location on the heart of the area heated or cooled was an important determinant of the magnitude of the changes produced, and of the lead in which this change was best exhibited. This question will be reported in greater detail in another communication.

Further proof is here afforded that the electrograms we have recorded are in reality the electrograms of the ventricle not treated with potassium chloride, for heating and cooling the treated ventricle produced no changes in the recorded waves, while heating and cooling the opposite ventricle produced marked changes. These results can only mean that the electrocardiograms thus recorded are derived from the untreated ventricle.

These findings lead to the conclusion that alterations in the T wave result from variations in the duration of the electrograms of each ventricle. As seen in figure 4, variations in the contour of the dextro- and levocardiograms were also produced by changes in temperature. These must also be considered to have contributed to the final form of the T wave.

CONCLUSIONS

1. The T wave results from the interference of the terminal portions of the dextro- and levocardiograms.
2. Heat curtails the dextro- and levocardiogram.
3. Cold prolongs the dextro- or levocardiogram.
4. Prolongation of the dextrocardiogram or shortening of the levocardiogram causes an upright T wave.
5. Shortening of the dextrocardiogram or lengthening of the levocardiogram inverts the T wave.

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THE SKIN TEMPERATURE OF HYPERTENSIVE RABBITS AND THE PRESSOR EFFECTS OF HEATED KIDNEY EXTRACTS[1]

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It has been shown previously in normal rabbits (1) that suitably heated kidney extracts elevate blood pressure conspicuously without diminishing blood flow to the ear, as indicated by the constancy of skin temperature during the entire pressor response. In sharp contrast, minute doses of epinephrine, tyramine, guanidine and pituitrin uniformly reduce peripheral blood flow whenever blood pressure is increased even slightly. Prinzmetal and Wilson (2) and Pickering (3) had found previously that in human hypertension peripheral blood flow in the extremities is approximately normal. Thus it appears that of the substances so far studied some component of kidney extract (presumably renin) is the only one which can imitate, though briefly, the circulatory changes characteristic of the hypertensive state in man. This interesting similarity made it seem desirable to extend these studies to rabbits made hypertensive by the Goldblatt method.

The experiments now described compare normal and hypertensive rabbits with reference to *a*, the initiation of peripheral vasodilatation by body warming; *b*, maximal skin temperature of the ear during complete vasodilatation as a rough measure of peripheral blood flow, and *c*, the effects of kidney extract with respect to the magnitude of the pressor response and the constancy of skin temperature during the period when blood pressure is elevated.

MATERIAL AND METHODS. All observations described here were carried out on male, white, New Zealand rabbits, weighing between 2.0 and 3.0 kilos, and fed on a standard adequate ration. To produce hypertension, small silver clips with a channel 0.4 to 0.6 mm. deep, were clamped about each renal artery through flank incisions. Before and after operation, blood pressures were measured in the central artery of the ear at weekly intervals by either or both of two methods; *a*, the recording oscil-

[1] The expenses of this study were defrayed in part by a grant from the Commonwealth Fund.

lometer previously described (1), or *b*, the simple capsule method developed by Grant and Rothschild (3). While blood pressure was being measured the vessels of the ear were kept widely dilated by raising the body temperature of the rabbit gradually to between 40.5 and 41.5°C. This was accomplished by wrapping an electric heating pad around the abdomen or, more usually, by placing the rabbit's body in a box with a double wall through which warm water (43–45°C.) was circulated. Rectal temperature and the skin temperature of the ear were both measured by the usual type of copper constantan thermal junction. The head and ears were exposed to room air at temperatures between 22° and 25°C.

Extracts of normal rabbit kidney tissue were prepared by suitable grinding, followed by heating to between 55 and 56°C. for twenty minutes, and subsequent filtration as described previously (1). These extracts were slowly injected intravenously in doses of 20 cc. at the rate of 1.6 cc. per minute for twelve minutes, through a minute T-cannula which had been stitched in a lateral ear vein at the beginning of the observation. It has been demonstrated (1) that with this technique salt solution or blood plasma could be injected into unanesthetized animals without disturbing skin temperature or blood pressure appreciably.

Observations. A. *Blood pressure levels in control and hypertensive animals.* Systolic blood pressures of normal animals observed as controls in these experiments generally ranged from 65 to 95 mm. of Hg; but in isolated animals readings as high as 103 mm. Hg were observed occasionally. After silver clips were applied to both renal arteries, approximately 60 per cent of the animals developed a sustained hypertension with systolic blood pressures between 110 and 176 mm. Hg; these will be referred to as "hypertensive animals." The silver clips used in these experiments were not adjustable so that if the clips, as originally applied, failed to produce hypertension the animals were necessarily discarded.

B. *Peripheral vasodilatation.* The vessels in the ears of normal and hypertensive rabbits responded similarly to changes in body temperature. When body temperature was between 37 and 39°C., the vessels of the ear were ordinarily constricted and the temperature of the skin at the tip of the ear approached that of the surrounding air (fig. 1). As body temperature was slowly raised the central artery of the ear dilated at first partially and spasmodically, but dilatation became complete when rectal temperature reached 40.5°C. or more. In a few minutes skin temperature reached a maximum and constant level which was lower than rectal temperature by between 1.5 and 4°C. This relationship held as long as the body temperature was kept above normal.

The larger section of figure 1 shows rectal and ear temperatures observed in a normal rabbit while body temperature was being slowly raised. The same figure could illustrate equally well the response of a hypertensive

rabbit to similar body warming. The inset in figure 1 shows ear temperature charted against rectal temperature. The solid line shows the average curve for ten normal rabbits; the dotted line shows the average curve for six hypertensive rabbits under similar conditions. At rectal temperatures

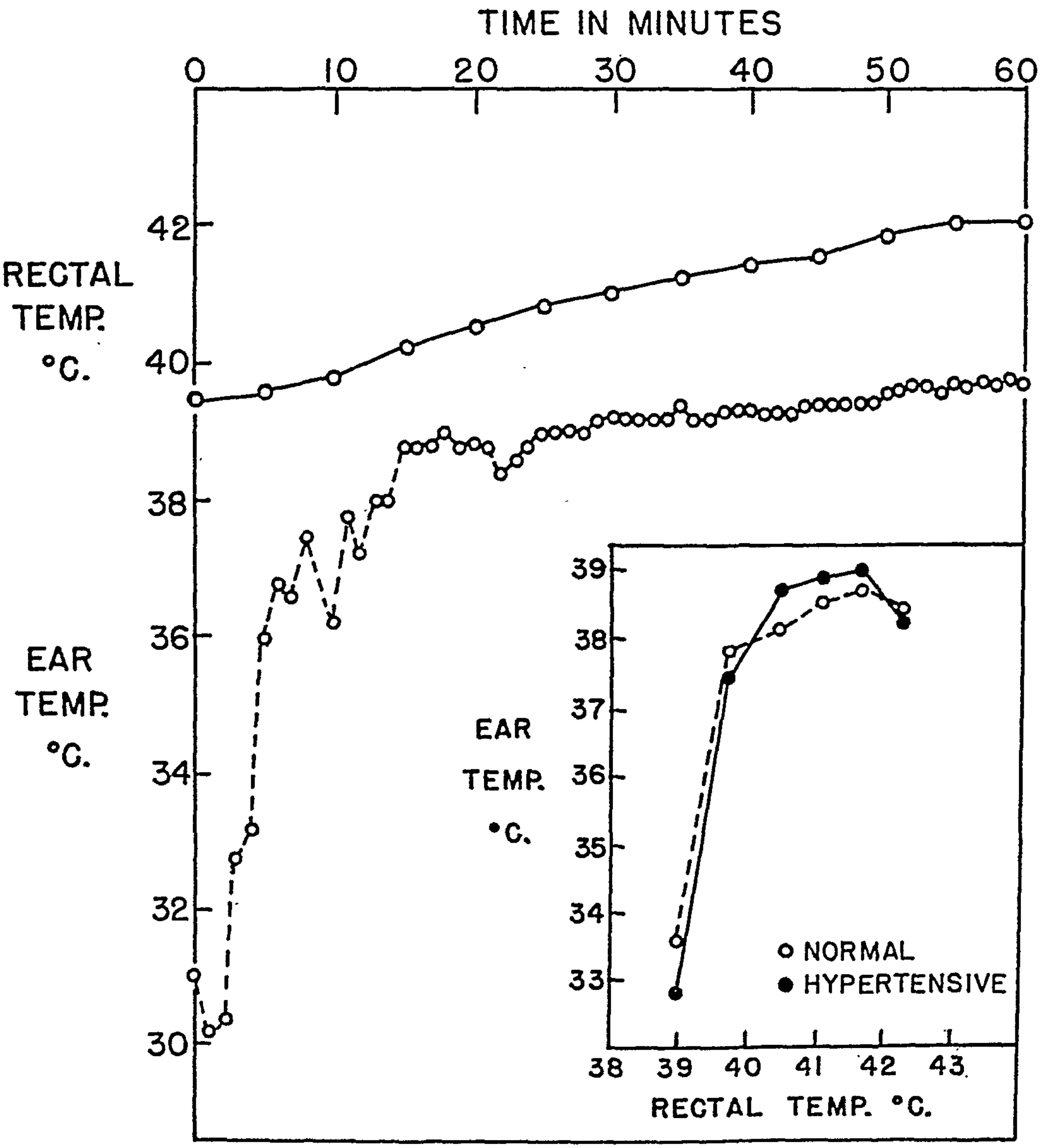

Fig. 1. Showing relationship of skin temperature of ear to rectal temperature in normal and hypertensive rabbits during body warming.

of 39.5 to 42°C. ear temperatures rose to similar maximum levels in the two groups, indicating that rabbits with experimental hypertension react normally to rising body temperature and that release of vasoconstrictor tone leads to a similar increase in peripheral blood flow. In this respect

a comparison of normal and hypertensive rabbits agrees with similar comparisons of peripheral blood flow in normal and hypertensive human subjects (2, 3).

The two curves are not identical, however, in that at rectal temperatures in the vicinity of 39°C. the average ear temperatures for the controls are slightly higher than those for the hypertensive animals. This is due to the fact that two of the hypertensive rabbits occasionally showed slightly delayed peripheral vasodilatation. The vessels did not relax until rectal temperature was between 40.3 and 40.7 instead of the more usual range between 39.5 and 40.3°C. Once dilatation began it rapidly became complete and the difference is so slight that it does not seem significant. Ill rabbits with normal blood pressure frequently show similar deviation from the normal reaction. In addition, it is possible that peripheral blood flow may actually be slightly greater than normal in the hypertensive animals during maximal vasodilatation. When rectal temperature was raised to between 40 and 41.5°C. the skin temperatures of the hypertensive rabbits were slightly higher than those of the normal rabbits under similar conditions. Thus, while it is safe to conclude that experimental hypertension in rabbits does not diminish blood flow to the ear, the method used does not exclude a slight increase in peripheral blood flow.

C. *The effects of kidney extract on blood pressure, skin temperatures and peripheral blood flow of hypertensive rabbits.* It has been shown (1) that the injection of a 10 per cent heated kidney extract into normal rabbits elevates systolic blood pressure by 40 to 45 mm. Hg without modifying skin temperature. Figure 2 illustrates a similar experiment using a hypertensive rabbit with a resting systolic blood pressure of 130 mm. Hg as the result of prior clamping of both renal arteries. The injection of 20 cc. of 10 per cent rabbit kidney extract into this animal elevated the blood pressure by over 50 mm. Hg, i.e., from 130 to 180 mm. Hg, also without modifying skin temperature. This result, typical of many additional observations, indicates that the pressor principle of kidney extract acts additively with the humoral mechanism of renal ischemia to elevate blood pressure still further. Both the persistent hypertension of renal ischemia and the added temporary elevation of blood pressure produced by kidney extract do not decrease peripheral blood flow measurably.

D. *Sensitivity of normal and hypertensive rabbits to kidney extracts of varying concentration.* It has been suggested frequently (5–7) that normal kidney tissue reduces the effect exerted by a given extract on the blood pressure of the recipient animal and, for this reason, nephrectomized animals have been recommended for assay purposes (5). In order to determine whether renal ischemia modified reactivity in rabbits, dilute and concentrated kidney extracts were injected into a series of normal and

hypertensive animals without anesthesia. Table 1 summarizes the results of this series of experiments.

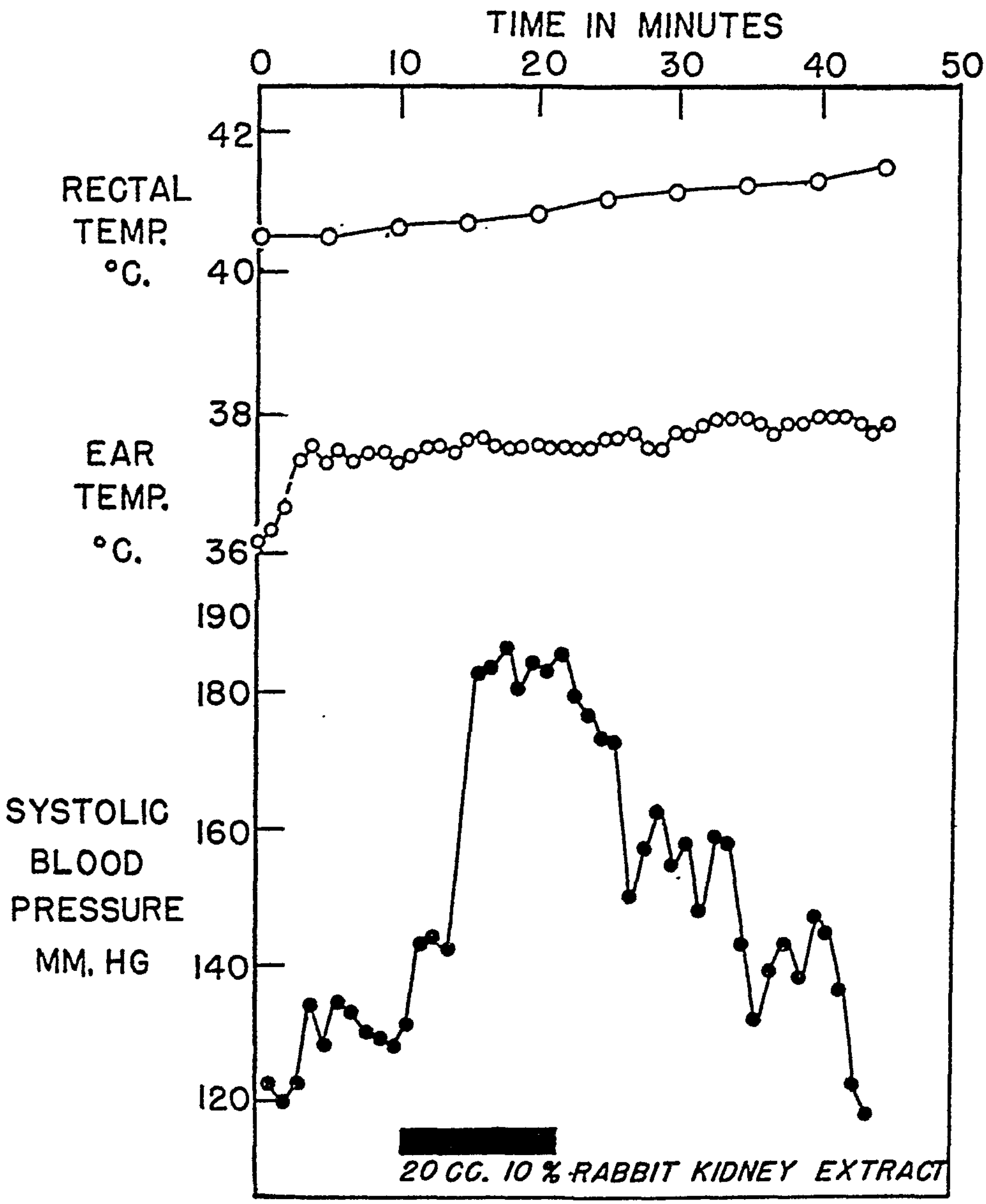

Fig. 2. Showing the effect of heated extract of normal kidney tissue of rabbit on systolic blood pressure and skin temperature during complete vasodilatation.

In neither group did blood pressure change significantly during the injection of 0.9 per cent NaCl solution and of 0.1 per cent heated rabbit kidney extract in the usual dose of 20 cc. injected at the rate of 1.6 cc. per minute over twelve minutes. Similar doses of 3.3 per cent rabbit

kidney extract elevated the blood pressure of the hypertensive animals more than that of normal animals, but when 10 per cent extracts were used the results became irregular, particularly in those animals with very high initial pressures.

TABLE 1

Showing changes in systolic blood pressure during injection of heated kidney extracts into normal and hypertensive rabbits

NORMAL ANIMALS			INJECTION: 20 CC. IN 12 MIN.	HYPERTENSIVE ANIMALS		
Blood pressure mm. Hg				Blood pressure mm. Hg		
Before injection	Maximum during injection	Difference		Before injection	Maximum during injection	Difference
78	78	0	NaCl 0.9%	134	132	−2
80	75	−5		110	110	0
90	89	−1		117	125	+8
75	78	+3		104	97	−7
70	70	0	Rabbit kidney extract 0.1%	130	130	0
87	90	+3		110	112	+2
78	80	+2		116	119	+3
				115	116	+1
81	95	+14	Rabbit kidney extract 3.3%	105	135	+30
77	85	+8		116	140	+24
84	100	+16		130	150	+20
83	138	+45	Rabbit kidney extract 10.0%	127	188	+61
88	138	+50		117	149	+32
74	119	+45		106	150	+44
70	102	+32		128	184	+56
				176	188	+12

DISCUSSION. Substances which raise blood pressure may be classified into two groups according to their effect on peripheral blood flow in the unanesthetized rabbit. Nearly all of the common pressor substances resemble epinephrine in reducing cutaneous blood flow conspicuously when injected in the smallest doses which elevate systemic blood pressure measurably. So far only two pressor substances have been found which do not decrease cutaneous blood flow when raising systemic blood pressure. One of these, renin, is found in extracts of kidney tissue while the second, alpha-N-dimethyl-p-hydroxyphenylethylamine sulphate (paredrinol sulfate) is a synthetic sympathomimetic drug allied to ephedrine. The latter according to Stead and Kunkel (8) produces in man a type of "hypertension which has many features in common with clinical hypertension," one of these features being the sustained normal peripheral blood flow.

The present studies indicate that the hypertensive rabbit also maintains a normal peripheral blood flow despite conspicuous elevation of systemic blood pressure. This finding adds to evidence accumulating from other directions indicating that experimental hypertension produced in animals by renal ischemia truly simulates the human hypertensive state in which peripheral blood flow is also practically normal (2, 3). Moreover, heated kidney extract injected into animals already hypertensive, and more reactive than normal to renin, raised blood pressure to still higher levels without decreasing peripheral blood flow.

In hypertensive rabbits small doses of heated kidney extract elevate systolic blood pressure by slightly greater absolute amounts than is the case when similar doses are injected into normal animals. These results agree with those of Katz and Friedberg (6) for hypertensive dogs and of Williams, Wegria and Harrison (9) for hydronephrotic rats with hypertension. Concentrated extracts in three of five experiments on hypertensive rabbits raised systolic blood pressure to maxima of 188, 184 and 188 mm. Hg, whereas the absolute elevations of systolic blood pressure varied widely and were sometimes less than the elevations produced by the same extracts in normal animals. It is possible that the pressor effect of renal ischemia combined with that of the kidney extract approached the limit of the vascular system to respond and that this masked the greater sensitivity that could be demonstrated with more dilute extracts.

SUMMARY

The cutaneous vessels in the ears of hypertensive rabbits and normal rabbits responded similarly, both quantitatively and qualitatively, to body warming. The hypertensive state induced by renal ischemia was not associated with measurably diminished peripheral blood flow, indicating another resemblance between experimental hypertension in animals and the hypertensive state in man.

Heated kidney extracts, injected into hypertensive rabbits, raised blood pressure to extremely high levels likewise without diminishing skin temperature. Apparently the temporary pressor effect of kidney extract is added to the more permanent hypertension due to renal ischemia, without diminishing peripheral blood flow.

When the same kidney extracts were injected into normal and hypertensive rabbits, the rise in blood pressure was slightly greater in the hypertensive group, except that with large doses in markedly hypertensive animals there appeared to be a maximal value of systolic blood pressure which could not be exceeded. Under these circumstances the blood pressure of the hypertensive rabbits was increased by absolute amounts which were less than those observed with the same extract in normal rabbits.

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SOME AFFERENT DIENCEPHALIC PATHWAYS RELATED TO CORTICAL POTENTIALS IN THE CAT

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Electrical responses to afferent stimulation have recently been described in the cerebral cortex. These responses have been obtained after stimulation of the sciatic nerve (Derbyshire *et al.*, 1936; Forbes and Morison, 1939), the saphenous nerve (Heinbecker and Bartley, 1940) and tactile sensory organs (Marshall, Woolsey and Bard, 1937). At least three types of response may be distinguished on the basis of latency, threshold and localization in the cortex. In Marshall, Woolsey and Bard's observations on the monkey the latency was 8 to 10 msec., and the effect was sharply localized in the sensory cortex. Forbes and Morison (1939) obtained two cortical responses to stimulation of the cat's sciatic with single shocks. An initial "primary" response whose latency was 8 to 10 msec. was followed by a "secondary" response 30 to 80 msec. after the stimulus. The voltage of the primary response was greatest in the contralateral sensorimotor cortex, but the activity was not localized sharply under the conditions of their experiments. The secondary response was obtained equally well in all regions of the cortex, on both the contralateral and ipsilateral sides.

The effects described above are obtained best under such deep barbiturate anesthesia that only slight spontaneous cortical activity appears in the records. They may be recognized, however, when a moderate degree of spontaneous activity is present. Indeed, Heinbecker and Bartley (1940) described cortical responses whose latencies are similar after stimulation of the saphenous nerve in unanesthetized cats. In addition, they describe a third response of still longer latency (400 msec.) which occurs only after stimulation strong enough to activate the C fibers of the nerve.

The present investigation was undertaken to determine, if possible, the course and location of the afferent pathway or pathways to the cortex involved in the production of the primary and secondary responses. Data will be presented in the following sections which indicate that different pathways are involved for the two responses.

Method. Cats were anesthetized with a preliminary dose of nembutal (0.7 cc. per kgm.). Later, more nembutal was added intravenously until

good secondary responses were obtained. The cerebral cortices were exposed and one or both sciatic nerves were prepared for central stimulation. Wick electrodes, with an interpolar distance of 3 to 10 mm., were placed on various regions of the cortex. For more precise localization, bipolar silver wire electrodes with a separation distance of approximately 1 mm. were used in some cases.

The electrodes were connected with the input of 5 push-pull stages of condenser coupled amplification, and the responses were recorded by a Grass ink-writing electroencephalograph. In various experiments 1 to 5 independent channels of amplification and recording were used.

The sciatic nerves were stimulated by single condenser shocks, discharged through a thyratron tube which was controlled in turn by a manually operated key. The stimulus artifacts were regulated by an impedance balance consisting of a potentiometer shunted across the stimulus leads and connected to ground through the center tap.

Lesions, the exact nature of which will be described in the text, were produced by section, excision, or removal by means of a suction pipette of different parts of the brain. Responses were tested in all cases before and after the operation. Whenever any responses were abolished by the lesions, tests were made repeatedly at increasing intervals of time in order to determine whether or not the response was only temporarily depressed. In all, 50 experiments were performed.

At the end of each experiment the brain was removed and fixed in 10 per cent formalin. After sufficient hardening, gross sections were examined in order to determine the nature and location of the lesions. In certain experiments Nissl sections were prepared (fig. 3).

Results. In 21 experiments responses were tested in both cortices after stimulation of both sciatic nerves separately. "Primary" responses whose latencies were 8 to 10 msec. were routinely obtained in 9 of these experiments in which the recording electrodes were located on the leg area of the contralateral sensorimotor cortex (figs. 4 and 6). Small primary responses also were obtained on the ipsilateral cortex in some of these animals, while in others no recognizable ipsilateral "primary" could be distinguished.

In the remaining 12 experiments in which the sciatics were stimulated the recording electrodes were placed on other regions of the cortex. Under these conditions a small primary response was sometimes recorded, but it was never as large as those recorded from the sensorimotor leg area. For any electrode position, moreover, the primary response was larger on the contralateral than on the ipsilateral cortex. It appears, therefore, that the primary response is localized in the sensorimotor cortex, and that its magnitude is greater on the contralateral than on the ipsilateral side.

"Secondary" responses whose latencies were 30 to 80 msec. also were

recorded in all 21 of the experiments in which both sciatic nerves were stimulated. These responses occurred generally throughout both the contralateral and ipsilateral cortices. The latencies of the effects could vary, depending upon the region from which they were recorded. When simultaneous recordings were made from frontal, parietal and occipital areas, the latency in the occipital and parietal areas was equal to, or longer, but never shorter than that in the frontal cortex.

The stimulus threshold for both the primary and secondary responses was the same as that of A fibers in the sciatic, when tested oscillographically. Furthermore, both primary and secondary responses appeared simultaneously when thresholds were determined with 4 stimulating capacities ranging from 1.0 to 0.01 μ FD. Lastly, in an experiment in which electrodes were placed on an uncut sciatic nerve, the primary and secondary responses appeared with a stimulus intensity which was liminal for the motor nerve fibers. It appears, consequently, that the primary and secondary responses are produced by stimulation of nerve fibers of the same group.

The secondary response appeared simultaneously in both cortices after stimulation of either sciatic. There are, therefore, both crossed and uncrossed components in the afferent pathways. The following experiments were undertaken to delimit the levels of crossing.

Hemidecerebration at the intercollicular level was performed in 4 cats. After removal of 1 hemisphere, secondary responses were obtained from the remaining cortex, when either sciatic was stimulated (fig. 1). It may be inferred, therefore, that there is a crossed afferent component below the intercollicular level.

In 7 cats the brainstem was hemisected with a sharp spade at the intercollicular level. The hemispheres were not disturbed otherwise. Following the hemisection the primary response was abolished on the side of the lesion, while secondary responses were recorded from both cortices after stimulation of either sciatic in 4 animals. In the 3 remaining animals only one sciatic was stimulated. Secondary responses were recorded from both cortices in the 3 cases. These experiments indicate that a crossed afferent path is present above, as well as below, the intercollicular level.

The parietal and occipital cortex, diencephalon and midbrain were removed on one side in 6 cats. This preparation consisted essentially of hemidecerebration from the intercollicular level to the level of the optic chiasma but leaving intact the frontal cortex, the septal and preoptic areas and the anterior part of the corpus callosum. In all 6 experiments, secondary responses were recorded from both frontal cortices after stimulation of either sciatic, indicating that the crossed afferent component exists anterior to the level of the chiasm (fig. 2).

In 2 preparations of the type described above, the corpus callosum was

cut in addition to the cortical, diencephalic and mesencephalic lesion. The secondary response disappeared on the side of the defect, but was recorded on the opposite normal side in both cases. Conversely, in another preparation with the frontal cortex isolated but for its midline connections, the structures beneath the corpus callosum were divided in the midline by section with a sharp spade, care being taken to guide the spade along the inferior margin of the callosum. Following this procedure, secondary responses were recorded from both frontal cortices (fig. 2). Subsequent anatomical observation revealed that in this case the frontal cortex was indeed isolated except for its connections through the corpus callosum.

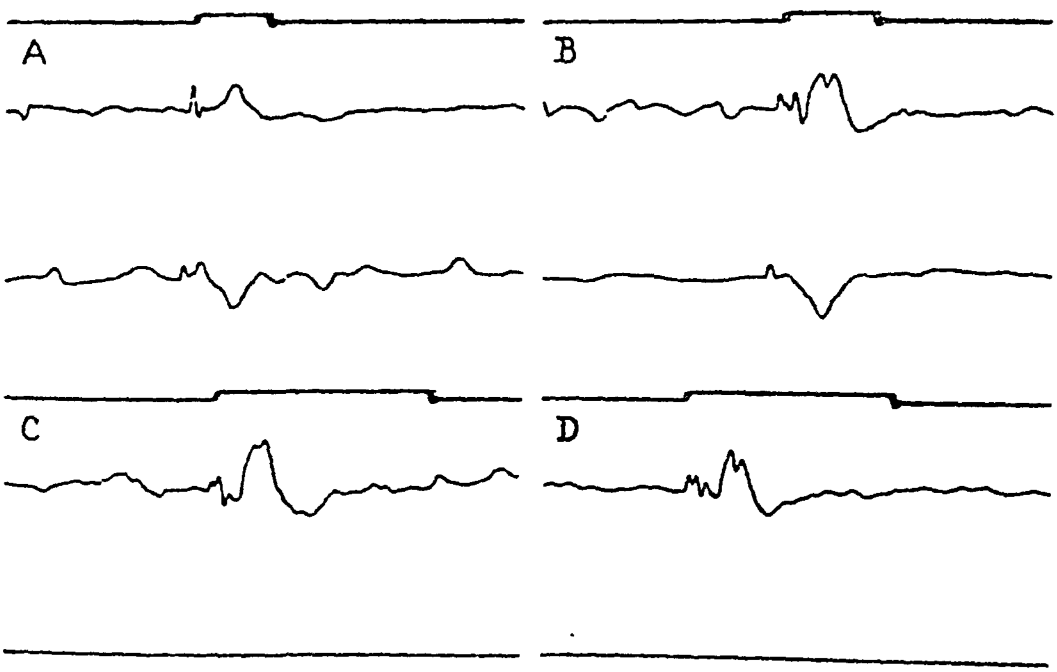

Fig. 1. Cortical responses to sciatic stimulation. Upper line, signal for stimulation; middle line, left sensorimotor cortex; lower line, right sensorimotor cortex. A and C, single shock stimulus to left sciatic; B and D, stimulus to right sciatic. Between B and C, the right hemisphere was removed from the intercollicular level forward. Paper speed—60 mm. per second.

In additional experiments on this point, the brainstem was hemisected at the intercollicular level and the corpus callosum was cut in 2 animals. Section of the callosum abolished the responses on the side of the hemisection, while secondary responses were recorded on the opposite, normal side. In another experiment the order in which the lesions were produced was reversed. The corpus callosum was first divided, and secondary responses were recorded in both cortices. Hemisection of the brainstem at the intercollicular level was then performed. After this lesion the responses were abolished on the side of the transection but were recorded on the opposite side.

Taken as a group, the experiments described in the preceding two paragraphs indicate that the upper crossed afferent path for the secondary response runs through the corpus callosum. Certain aspects of this pathway will be discussed in a separate communication. In order, however, to determine the course of the afferent path which is uncrossed above

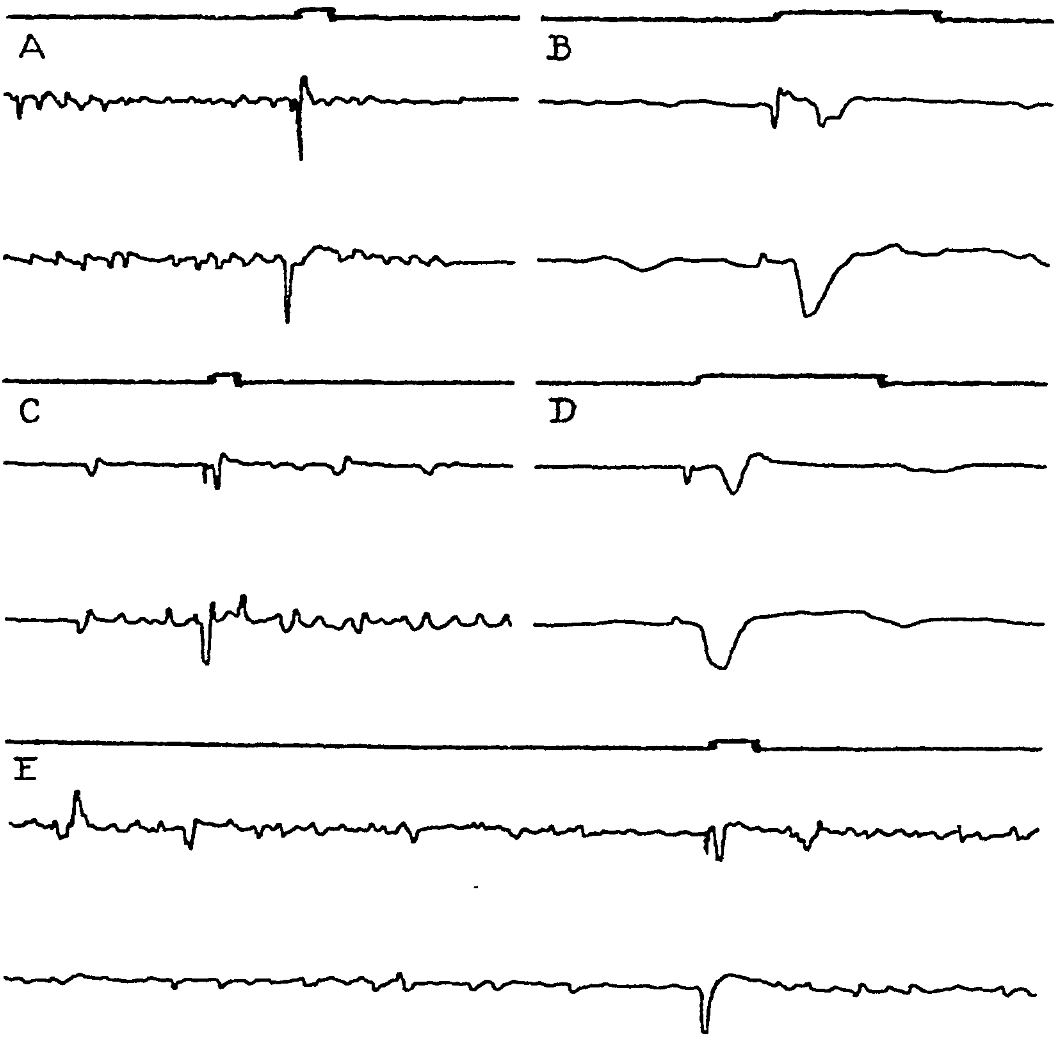

Fig. 2. Records as in figure 1. All stimuli to right sciatic nerve. A and B, normal brain. C and D, after left hemidecerebration, leaving intact the frontal cortex (see text). E, after midline sagittal section of all structures beneath the corpus callosum. Paper speeds, A, C and E, 15 mm. per sec. B and D, 60 mm. per sec.

the level of the colliculi, experiments were made in which unilateral defects were produced together with section of the corpus callosum, or in which bilaterally symmetrical lesions were placed.

In 5 cats the thalamus was removed or damaged extensively on one side (fig. 3). The operative procedure was used successfully to prepare chronic athalamic animals. A cut was made through the cortex into the ventricle,

2 to 5 mm. behind and parallel to the ansate sulcus. After identification of the lateral ventricle, the cortex overlying the thalamus was elevated and cut out. The cortical tissue which was removed consisted of those parts of the marginal gyrus, the gyrus fornicatus and the medial part of the suprasylvian gyrus which lie behind the anterior limit of the defect. The hippocampus and fornix, which were exposed by the cortical defect, were next removed, bringing into view the superior aspect of the thalamus.

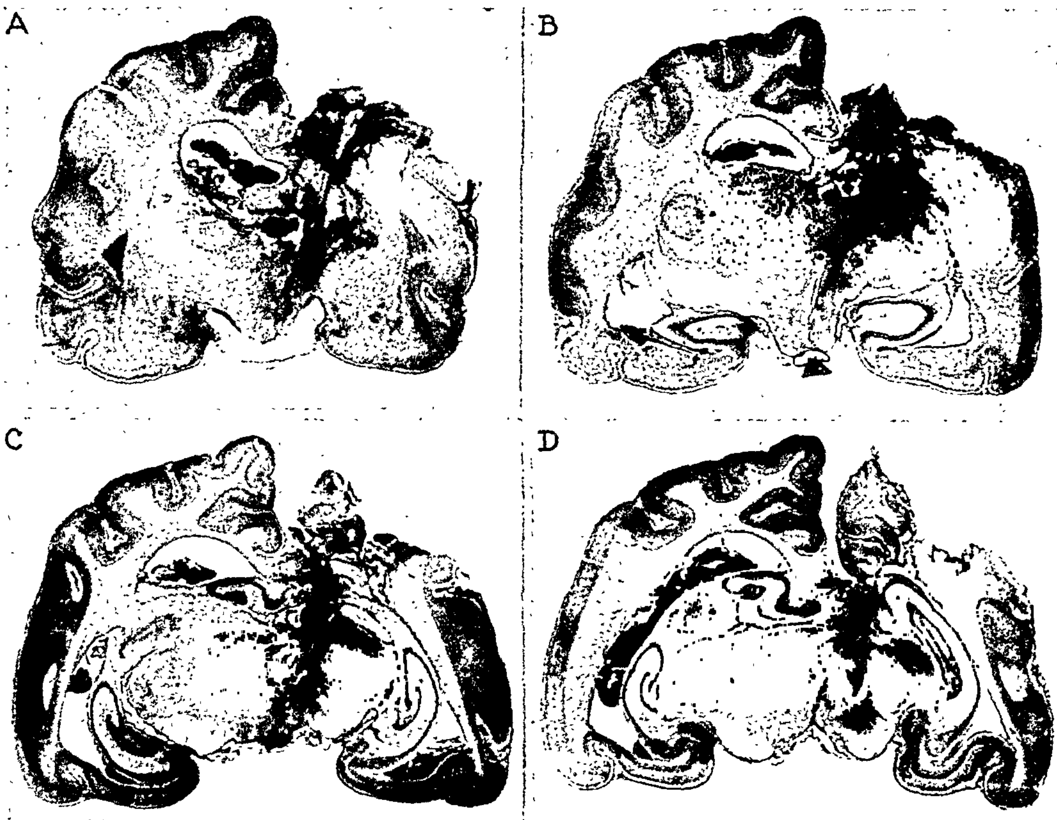

Fig. 3. Nissl sections through the level of the lesion in a cat from which the left thalamus had been removed three weeks previously. Cortical responses to sciatic stimulation in this animal are illustrated in figure 4. A, section through posterior border of optic chiasma and the anterior tubercle of the thalamus. B, section through the tuber cinereum and the anterior part of the lateral geniculate body. C, section through the mammillary bodies and posterior part of the lateral geniculate body. D, section through the red nucleus and the medial geniculate body.

Cuts were then made separating the massa intermedia in the midline and through the middle of the lateral geniculate body in the frontal plane. The body of the thalamus was removed by scooping or by suction. In all cases particular care was taken to remove the nucleus ventralis pars externa which is known (Ranson and Ingram, 1932) to receive fibers from the leg division of the medial lemniscus. Throughout the operative procedure close attention was given to hemostasis. Immediately after

the thalamic lesion the spontaneous electrical activity of the cortex was similar to that observed in chronic athalamic animals. Electrical stimulation of the motor cortex was followed by movements in most of the animals studied, indicating that the pyramidal system was still intact and functional.

Secondary cortical responses were recorded from both sides in these 5 unilaterally athalamic cats after stimulation of either sciatic (fig. 4, A

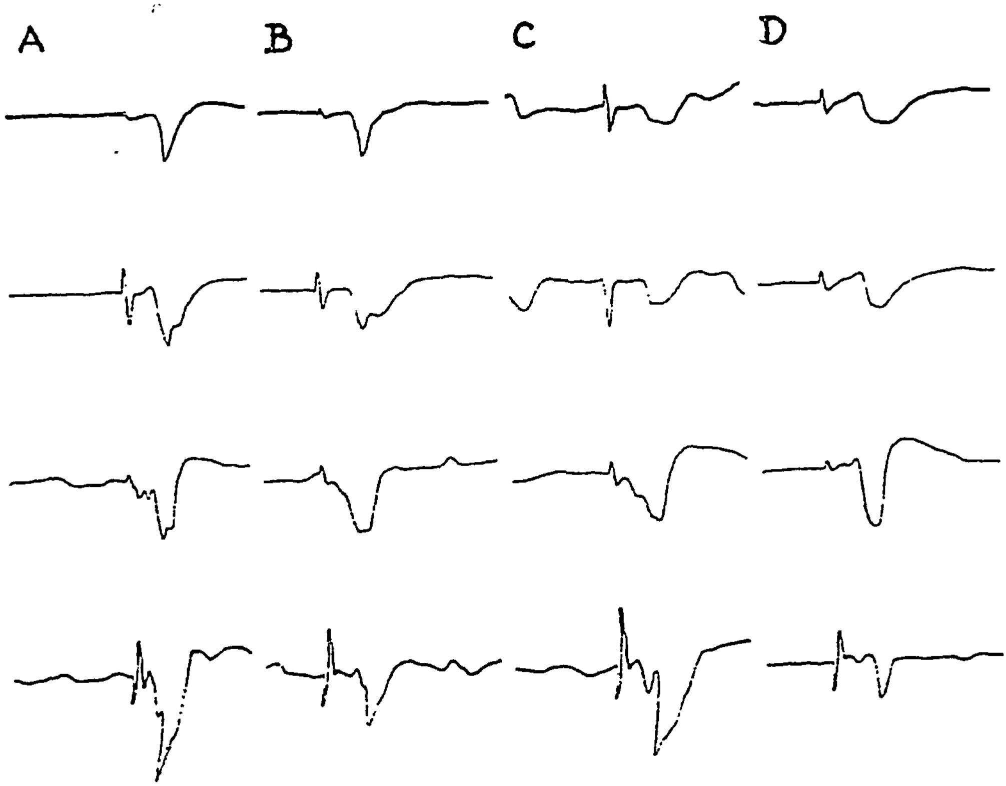

Fig. 4. Cortical responses after removal of thalamus. Records from above downward, left anterior sigmoid gyrus, left posterior sigmoid gyrus, right anterior sigmoid gyrus, right posterior sigmoid gyrus. Stimulations, A and C, left sciatic; B and D, right sciatic. The left thalamus had been removed three weeks previously (see fig. 3). Primary responses are seen in the right cortex, not in the left. Secondary responses are present in both cortices. Between B and C the corpus callosum was cut. Paper speed, 60 mm. per sec.

and B). In one of the experiments the recording electrodes were placed on the sensorimotor leg area in order to record both primary and secondary responses. After the operation, no primary could be detected in the record from the operated side.

The corpus callosum was then sectioned in these 5 animals. Bilateral secondary responses were recorded from 4 of the 5 (fig. 4, C and D). In the fifth, no response to sciatic stimulation could be detected on the operated side.

In 2 animals, after unilateral removal of the thalamus, the opposite thalamus was sucked out through the midline exposure. Secondary responses were recorded from both cortices in 1 of these animals, while in the other no cortical responses to stimulation were present. These experiments indicate that the uncrossed afferent pathway does not run through the thalamus, at least so far as the ventral, the medial or the anterior groups of nuclei are concerned.

Lesions also were made in the medial lemniscus at its entrance into the thalamus. The corpus callosum was sectioned and the lateral division of

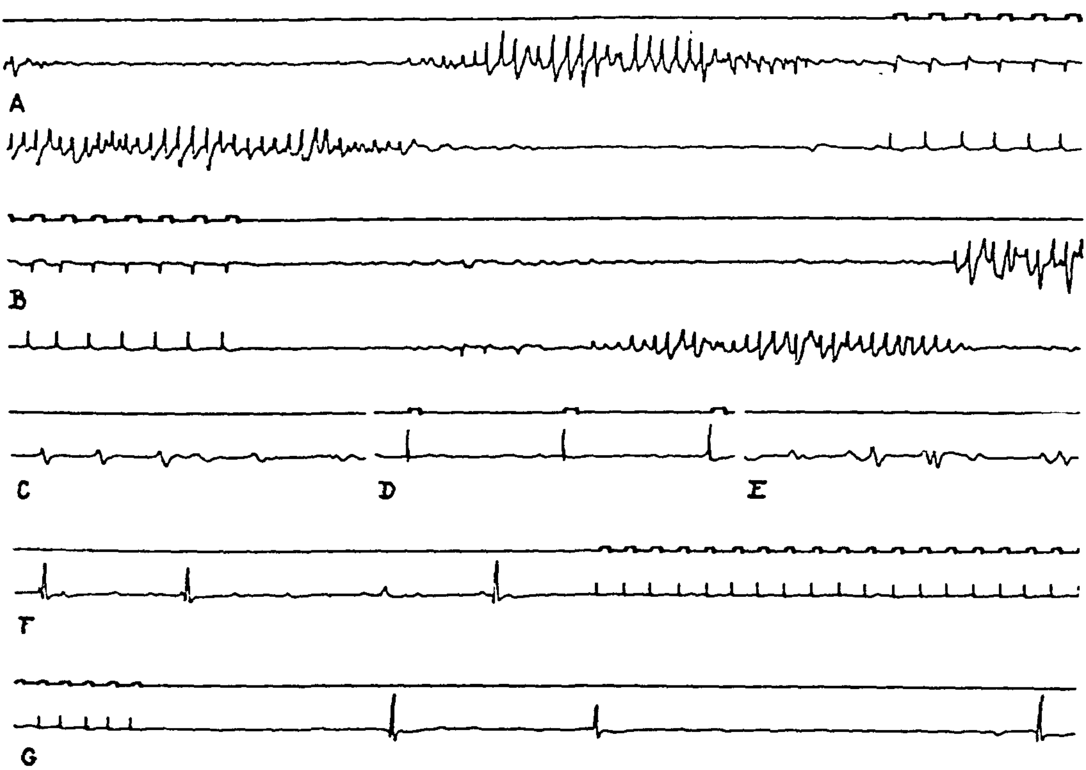

Fig. 5. Inhibition from sciatic stimulation. Records as in figure 1. Between A and B, 61 seconds of record removed. C, after bilateral cuts in the midbrain, sparing the central structures (see text). D and E, during and after sciatic stimulation. F, after application of strychnine to the cortical tissue. Between F and G 38 seconds of record removed. Paper speed, 7.5 mm. per sec.

the medial lemniscus cut on one side in 3 animals. Secondary responses were recorded from both cortices in 2 of these animals. In the third, no cortical response to sciatic stimulation was detected.

The lateral division of the medial lemniscus was sectioned bilaterally in 3 cats. Although no primary responses were seen in any of the 3 animals after the operation, the secondary effects were readily recorded in 2 cases. In the third, both primary and secondary responses were abolished after the section, but stimulation of either sciatic led to a suppression of the spontaneous and strychnine induced electrical activity of the cortex

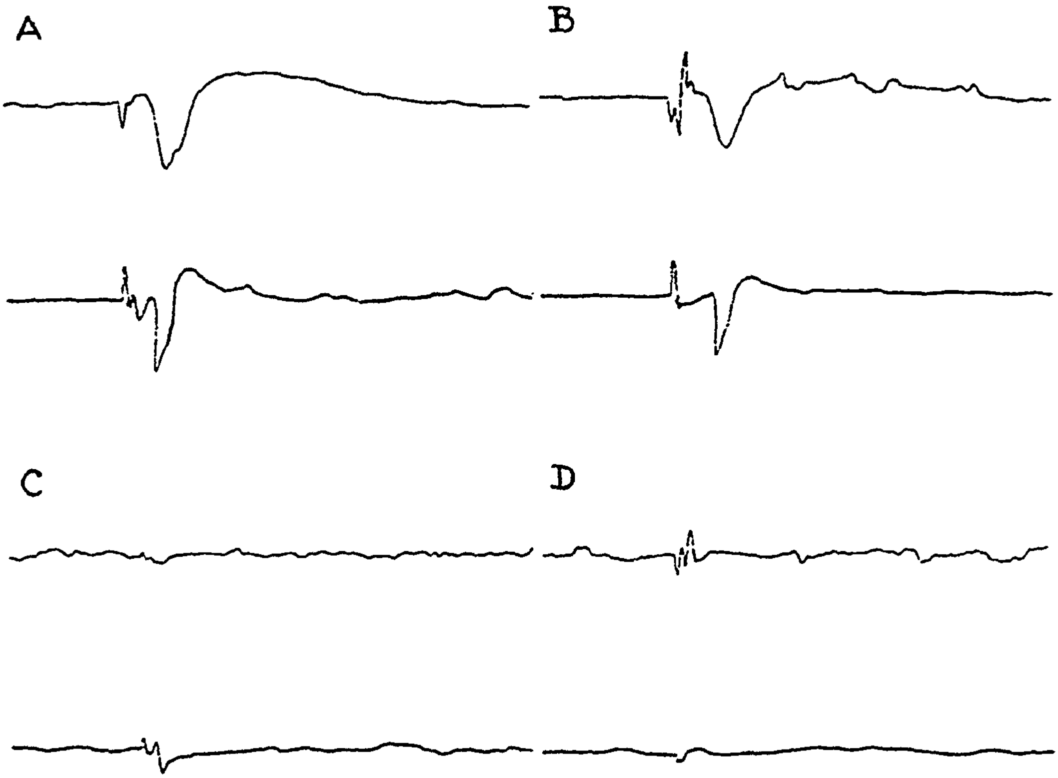

Fig. 6. Upper line, left posterior sigmoid gyrus; lower line, right posterior sigmoid gyrus. Stimulations, A and C, left sciatic; B and D, right sciatic. Both primary and secondary responses are seen. Between B and C, the lesion shown in figure 7 was placed. The primary response is still seen, but the secondary response has been abolished. Paper speed, 60 mm. per sec.

Fig. 7. Photograph of section through a midline lesion in the brain stem. Cortical responses to sciatic stimulation in this animal are illustrated in figure 6. The primary response was present and the secondary response was abolished after the lesion was placed.

(fig. 5). This inhibition was most marked when the sciatic was stimulated with a series of shocks at frequencies of 1 to 4 per second. Similar inhibition of spontaneous activity has been seen occasionally in normal animals after stimulation of the sciatic with slow frequencies (fig. 5).

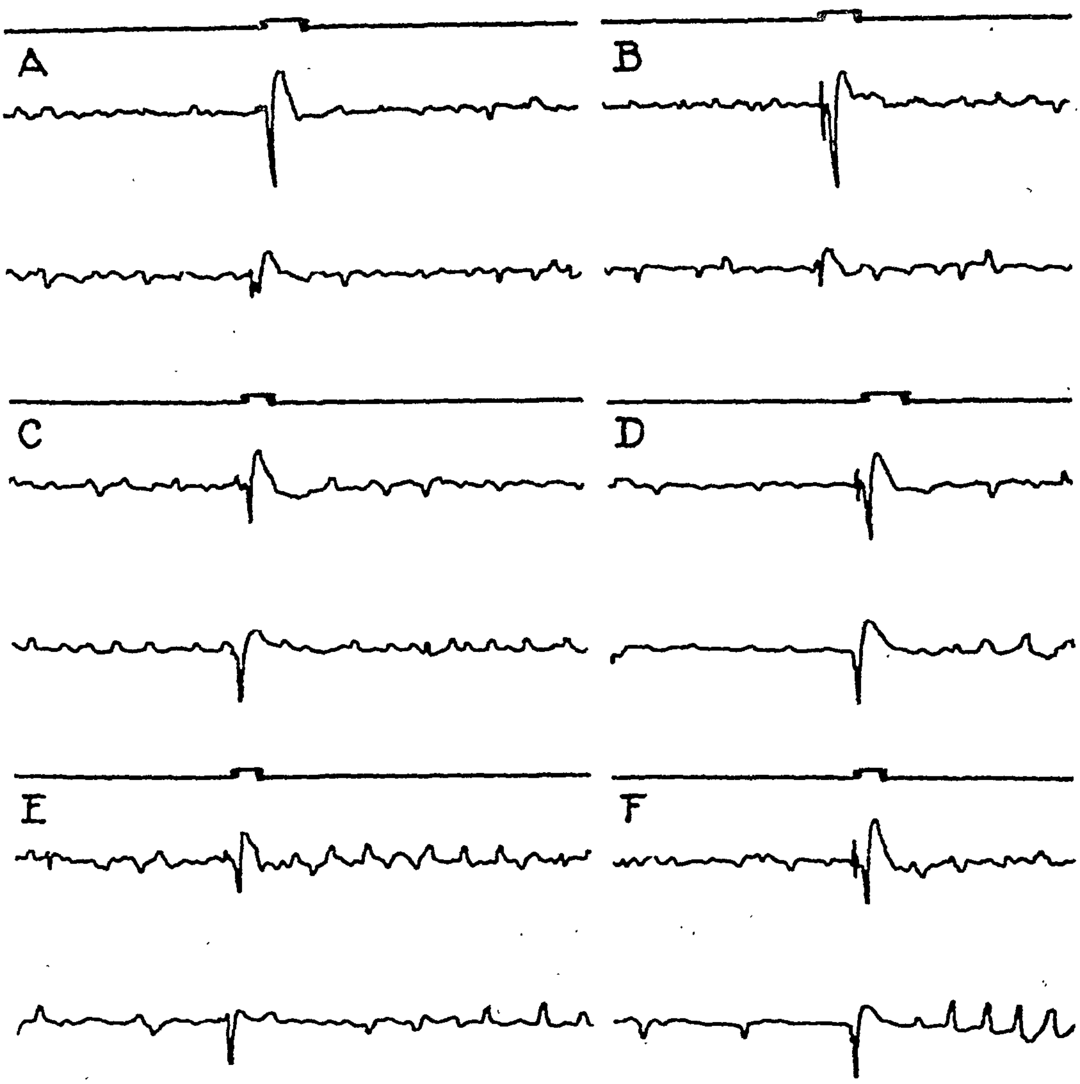

Fig. 8. Records as in figure 1. Stimulations, A, C and E, left sciatic; B, D and F, right sciatic. A and B, records from normal animal. C and D, after section of the cervical sympathetic nerves. E and F, after removal of the cerebellum. Paper speed, 15 mm. per sec.

The next type of experiment is the converse to that in which the medial lemniscus was sectioned bilaterally. In 4 cats a symmetrical cut was placed in the midline structures, beginning dorsally in the pretectal area and slanting slightly forward, to end ventrally at the level of the tuber cinereum. The lateral edges of the lesion involved the medial part of the

medial lemniscus but spared its lateral division which at this level has swung quite far outward as it enters the thalamus (fig. 7). In all 4 experiments good primary responses were present after the operation (fig. 6). In 3 of the 4, the secondary response was abolished by the lesion while in the fourth, both primary and secondary responses were obtained. Examination of the brain of this animal showed that the cut had been made further forward than had been intended and that the lesion was not entirely symmetrical in the subthalamic region.

In view of the long latency of the secondary response it was considered that it might represent vascular changes mediated by the sympathetic system. Another possibility was that it could be due to the activation of cerebellocortical connections. The cerebellum is known to receive spinal afferents, and the delay could be accounted for in the cerebellar circuits. The two possibilities were tested in the following experiment. Both cervical sympathetic nerves were sectioned in one animal, and secondary responses were subsequently recorded in both cortices after stimulation of either sciatic. The cerebellum was then excised. Secondary responses were still recorded in both cortices (fig. 8). The appearance of the secondary response, therefore, does not require either sympathetic or cerebellar circuits.

Discussion. At least three types of electrical responses may be produced in the cerebral cortex by stimulation of the sciatic nerve. The primary response appears with a latency of 8 to 10 msec. under the conditions of our experiments, and is localized in the leg area of the sensorimotor cortex. It is greatest in magnitude in the cortex contralateral to stimulation, but may also be present in the ipsilateral side.

The secondary response appears with a latency of 30 to 80 msec. and is not localized in any cortical region. Its latency, however, seems to be greater in the occipital than in the frontal cortex. These results are in agreement with those of Forbes and Morison (1939).

In addition to the above responses, inhibition of spontaneous cortical activity occurs after sciatic stimulation in certain circumstances. The exact conditions which are necessary for demonstration of this effect are not known. When present, inhibition is most prominent with stimulation frequencies which are fairly slow (1 to 4 per sec.).

These 3 cortical responses are not only different in character, but are produced by different afferent mechanisms. The primary response is abolished by lesions in the thalamus or lateral division of the medial lemniscus, while the secondary response remains after these procedures (fig. 4). Conversely, the secondary response is abolished by midline and subthalamic lesions at the level of the posterior border of the thalamus, while after these defects the primary response usually persists (fig. 6).

Finally, in the single experiment in which the brainstem had been damaged laterally on both sides, leaving intact only the central structures, inhibition of spontaneous activity could be demonstrated, although both primary and secondary responses had been abolished (fig. 5). It appears, therefore, that the afferent fibers for these three responses are to be found each in a different region, and that the responses may be selectively abolished by properly placed lesions.

The afferent to the primary response runs through the lateral part of the medial lemniscus, and projects to the leg area of the sensory cortex through the lateral nuclei of the thalamus. This conclusion is borne out by experiments (Morison, Dempsey and Morison, 1941) in which stimulation of the ventrolateral thalamic nuclei and the thalamic radiations produced cortical responses similar in all respects to the primary response. The latency and localization of this response are entirely similar to the potentials reported by Marshall, Woolsey and Bard (1937) after tactile stimulation. Moreover, since these regions are known to subserve functions of touch and proprioception, it is reasonable to conclude that the primary response is associated with these functions.

The course of the afferent fibers to the secondary response is less clear. The fibers do not run through the lateral part of the medial lemniscus or through the ventrolateral nuclei of the thalamus, since ablation of these structures does not abolish the response. In the experiments in which the secondary responses were abolished after thalamic lesions, anatomical inspection of the brains showed that the defect had involved the subthalamus as well as the thalamus. It is possible, therefore, that the afferent fibers run forward through the subthalamus. Stimulation of the subthalamus (Morison, Dempsey and Morison, 1941) is followed by secondary responses in the cortex, a fact which fits with this hypothesis.

The localization of the afferent fibers which inhibit cortical activity is obscure. At present, it can only be said that inhibition has been demonstrated after symmetrical lateral cuts in the midbrain which abolish both primary and secondary responses. It would appear, therefore, that the afferent fibers must run forward through the midline structures of the mesencephalon.

It was suggested by Forbes and Morison (1939) that the primary response might represent the arrival of the sensory impulse at the cortical level. This primary response, it was postulated, might then be regarded as the stimulus which sets off the secondary response. The failure of the secondary response to follow rapidly repeated stimulation could be due then to a decline in magnitude of the primary to subthreshold value, or to inhibitory effects which also were set up by sciatic stimulation. The present experiments, in as much as they demonstrate that the primary

and secondary responses are independent and are set off by different afferent pathways, render untenable the hypothesis that the primary response is the trigger for the secondary response. The failure of the secondary response to follow rapidly repeated stimuli must be due, then, to a long recovery process or to co-existing inhibition. That sciatic stimulation may inhibit both spontaneous and induced cortical activity is demonstrated in figure 5.

Heinbecker and Bartley (1940) have described cortical potentials after sensory stimulation in unanesthetized cats. The time relations of these potentials, which are similar to those of the primary and secondary responses, are attributed to conduction to the cortex over fast and slow fibers respectively. The long latency of the secondary response in our experiments cannot be a result of slow afferent conduction, since both the primary and secondary responses have the same stimulation threshold, which (Cf. Gasser and Erlanger, 1937, p. 41) indicates activation of fibers whose conduction velocity is the same. Furthermore, experiments in which the brainstem was stimulated (Morison, Dempsey and Morison, 1941) have shown that the secondary response can be elicited with practically unchanged latency when the stimulating electrodes are in the subthalamic areas.

CONCLUSIONS

1. Stimulation of the sciatic nerve in cats deeply anesthetized with nembutal may produce three cortical effects. These are: a primary electrical response whose latency is 8 to 10 msec. and which is localized in the leg sensorimotor area; a secondary response whose latency is 30 to 80 msec. and which may be recorded from any cortical area; and finally, inhibition of spontaneous cortical activity.

2. The primary response is abolished by lesions which destroy the thalamus or the lateral division of the medial lemniscus (fig. 4), while the secondary response remains after these procedures. It is concluded, therefore, that the former response is associated with functions of touch or proprioception.

3. The afferent supply to the secondary response is both crossed and uncrossed below and above the level of the colliculi. The upper crossed component runs through the corpus callosum (fig. 2). The uncrossed upper component is abolished by lesions which destroy the subthalamus (fig. 6), but the response remains after destruction of the thalamus (fig. 4).

4. Inhibition of cortical activity may occur after bilaterally symmetrical lesions in the midbrain which abolish both primary and secondary responses (fig. 5). The afferent pathway for inhibition runs, therefore, through the midline structures in the midbrain.

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CORTICAL RESPONSES FROM ELECTRICAL STIMULATION OF THE BRAIN STEM

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Cortical action potentials are produced by sensory stimulation under relatively deep barbiturate anesthesia (Derbyshire, Rempel, Forbes and Lambert, 1936; Marshall, Woolsey and Bard, 1937; Forbes and Morison, 1939, cf. for references). The cortical responses may be classified on the basis of latency and localization in the cortex. Different types of cortical responses may be selectively abolished by discrete lesions in the brain stem and diencephalon, and it has been suggested, therefore, that different afferent pathways are involved in the production of the various types of response (Dempsey, Morison and Morison, 1941). The present paper deals with the further tracing of these paths with stimulating electrodes introduced into the brain substance.

MATERIAL AND METHODS. Cats, anesthetized with nembutal, were used in this study. The skull was opened, exposing both cortices, and one or both sciatic nerves were prepared for stimulation. A modified Horsley-Clarke stereotactic instrument was attached to the skull and used as a carrier for the stimulating electrodes. This instrument, designed by one of us (R. S. M.) in conjunction with Dr. D. McK. Rioch, consists of a light aluminum frame attached to the edges of the skull at three points through universal joints, and an electrode carrier which allows for measured movements in three rectangular planes fitted to the frame (fig. 1). Although the device cannot be satisfactorily substituted for the conventional Horsley-Clarke apparatus when precise orientation of an electrode through a small bone defect is desired, it possesses compensating advantages. Its small size permits easy access to the exposed brain for the purpose of carrying out extensive operative procedures or the placement of a large number of cortical recording electrodes. The light weight (42 grams) leaves the head free to move easily in any plane without inducing additional sensory stimulation, and the absence of ear plugs permits the instrument to be used in the study of responses involving the autonomic nerves (to the orbit and salivary glands) which run through the middle ear. The coördinate readings do not determine the position of the electrodes with the definitive accuracy of the conventional instrument, but

with a little experience, an application of correction factors for variation in skull shape enables a comparable precision in use.

Recording electrodes, consisting of cotton wicks soaked in Ringer's solution, were connected by Ringer-agar-silver chloride junctions to silver wires. These wires led, in turn, to 5 push-pull stages of condenser coupled amplification. Recording was accomplished by a Grass ink-writing electroencephalograph. Two to five separate channels of amplification were used in various experiments.

Stimuli were single condenser discharges led to the stimulating electrodes through a transformer. The condenser discharges were regulated

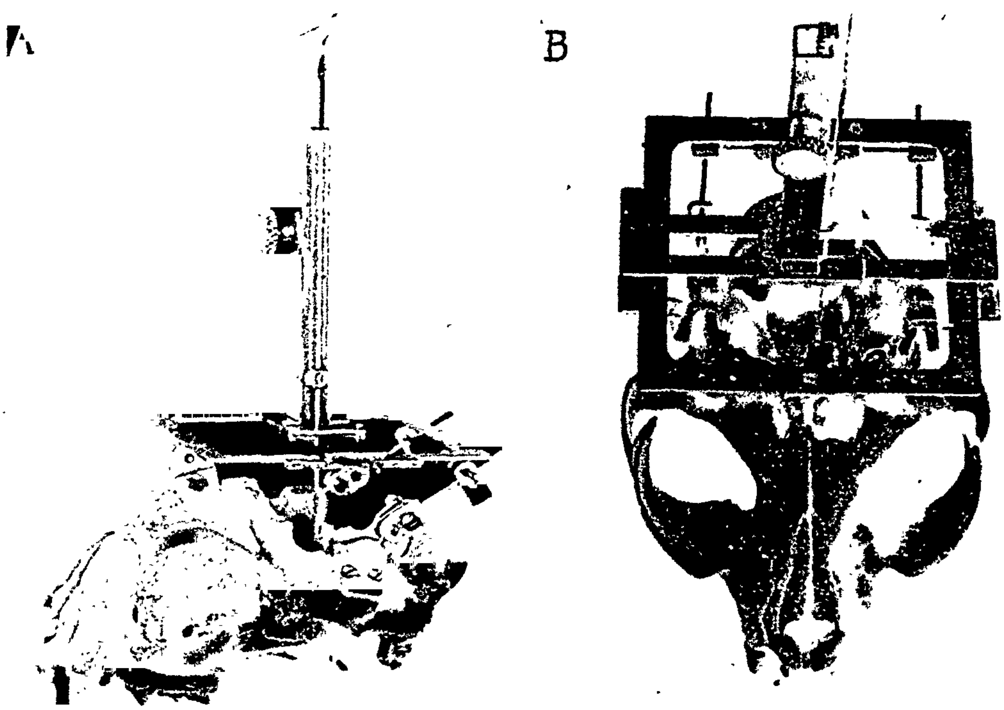

Fig. 1. Photographs of stereotactic instrument described in text, as applied to the skull of the cat.

by a thyratron tube which was controlled, in turn, by an external key. The stimulating electrodes consisted of 20-gauge steel tubing through which was placed an insulated copper wire. The steel tubing was covered, except at the tip, with an insulating layer of de Khotinsky cement. The center wire, also with bared tip, protruded about 1 mm. beyond the end of the tubing. Stimulation artifacts were controlled by a Wagner ground consisting of a potentiometer shunted across the stimulus and grounded at the center tap.

After preparation of the animal, more nembutal was injected intravenously until the base line of cortical activity was relatively stable and good secondary responses were obtained from sciatic stimulation (Forbes and

Morison, 1939). The stimulating electrodes were then introduced for systematic exploration of the brain stem and diencephalon. The explored region extended from the anterior commissure back through the level of the red nucleus. Because of the damage to the brain produced by the electrode tracks, all regions were not included in any single experiment, but overlapping regions were explored in different animals. In all, 1213 points were stimulated in the brains of 16 animals.

At the end of each experiment the brains were removed and fixed in formalin. For certain experiments Nissl sections were prepared for purposes of illustration. The shrinkage which occurs on embedding made it difficult to identify the points at which stimuli were applied. Consequently, in most of the experiments freehand sections of formalin-fixed material were made through the needle tracks, and the points were identified by gross measurements of the brains with reference to the coördinates of the stereotactic instrument.

Since the degree of localization provided by stimulation experiments is valid only in so far as it can be shown that the stimuli do not spread to regions remote from the electrodes, the following precautions against stimulus spread were taken. In the first experiments each point stimulated was explored routinely with stimulus intensities varying from zero to the maximum output of the thyratron stimulator. The threshold was determined for any response at any electrode position. The electrodes were then moved 1 mm. and the threshold for the same response again determined. In this way it was established that for each millimeter difference in electrode position there was a corresponding difference of approximately 20 points in the stimulus threshold. In all experiments the lowest threshold encountered was position 20 on the stimulus intensity dial.

In later experiments the procedure was modified as follows. The stimulus-intensity control was placed at position 30, corresponding to threshold plus 10 points. This position was chosen since it seemed likely that the stimulus spread would be confined to a sphere whose center was the electrode tip and whose radius was approximately one-half millimeter. Moving the electrodes downward millimeter by millimeter and stimulating at each point should therefore cover all regions in the electrode track and still permit localization to within 1 mm. In actual practice it appeared that this was accomplished. Responses obtained at one point usually were abolished by raising or lowering the electrodes 1 mm., and always were abolished when the electrodes were moved 2 mm. or more.

Results. Five types of cortical responses were produced regularly by stimulation in these experiments. These may be classified in the following manner. 1. A response whose latency is short (8 to 10 msec.) and which is localized more or less sharply in regions of the cortex on the side to which

stimuli were applied (fig. 2). 2. A fast response, similar to the one described above, which is succeeded by trains of spikes which are similar to the bursts of activity normally seen in the electroencephalogram of anesthetized animals (fig. 3). 3. A response of 30 to 80 msec. latency whose polarity is opposite to that of the prevailing spontaneous activity, and which may be recorded from all regions of both cortices (fig. 4, C).

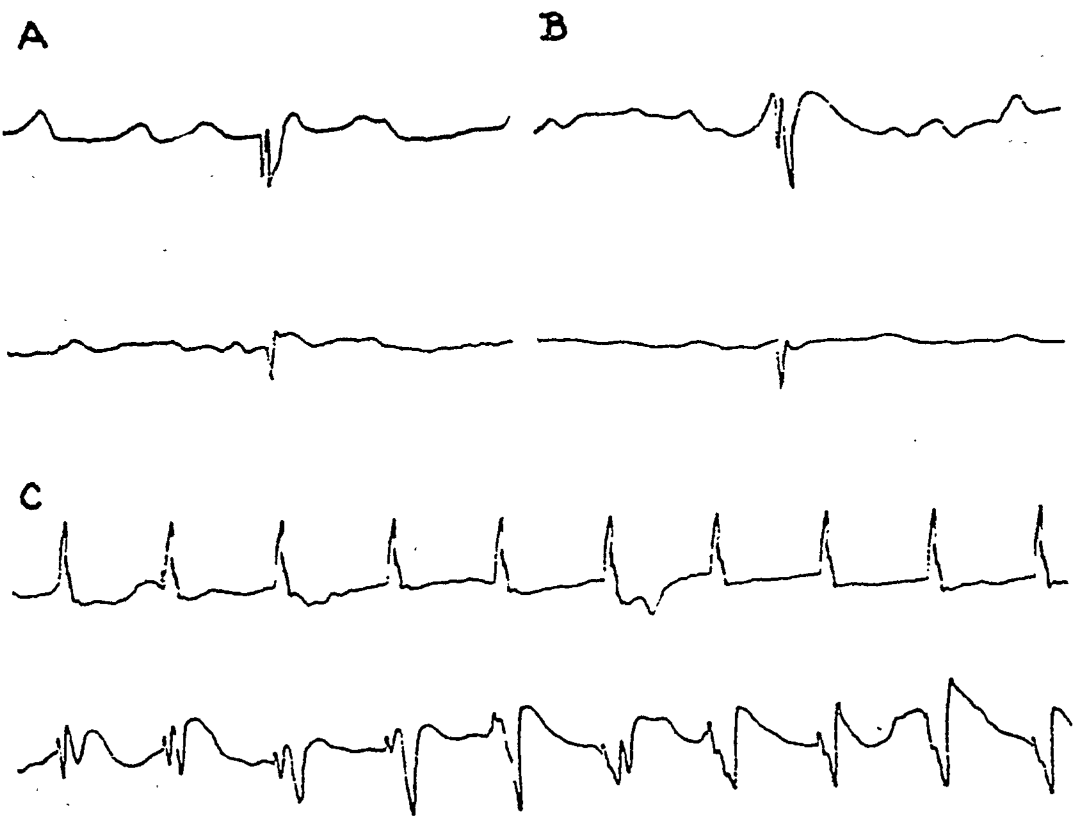

Fig. 2. Presence of short latency response ipsilateral to thalamic stimulation:
A. Electrograms of left (upper record) and right anterior sigmoid gyri. Shock artifact signals time of stimulation of left N. ventralis pars arcuata. The paper speed in this and succeeding records is 60 mm. per sec., unless otherwise noted.
B. Posterior sigmoid gyri of another preparation. Stimulation of left N. ventralis pars externa.
C. Recruitment of short latency response. Posterior sigmoid gyri of a third preparation. Stimulation of right N. ventralis pars externa at 5 per sec. Note: The upper tracing (left side) consists entirely of shock artifact.

This response is similar in all respects to the secondary discharge in the cortex after sciatic stimulation (Forbes and Morison, 1939; Dempsey, Morison and Morison, 1941). 4. A response which is similar in all respects to the secondary discharge, except that its latency is longer (100 to 250 msec., fig. 4, D). 5. Lastly, stimulation of certain areas in the brain leads to no visible action potentials, but rather causes the total or partial

suppression of the spontaneous activity which may be present at the time of stimulation (fig. 5).

A. *The short-latency, localized response.* The fast response (8 to 10 msec. latency) is encountered most frequently in the sensorimotor cortex and is seen only on the side to which stimuli are applied. It is produced by stimuli in the region of the lateral part of the medial lemniscus, in the ventral nuclei of the thalamus, and in the thalamic radiations (cf. figs. 2

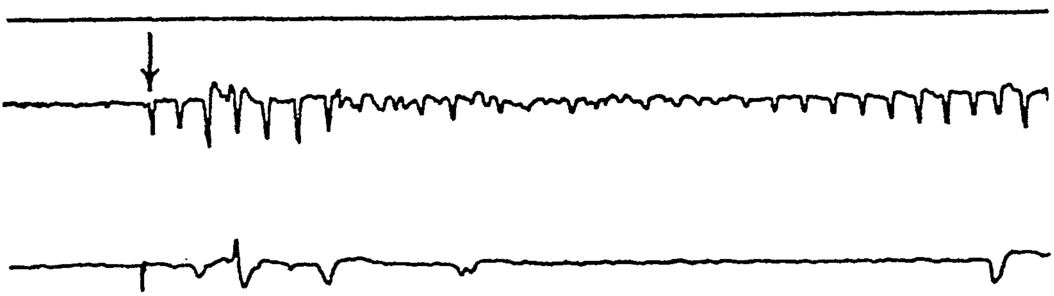

Fig. 3. Short latency response followed by repetitive bursts. Left (upper record) and right posterior sigmoid gyri. Arrow denotes single stimulus to anterior nuclear mass of thalamus (left). The activity on the right is spontaneous.

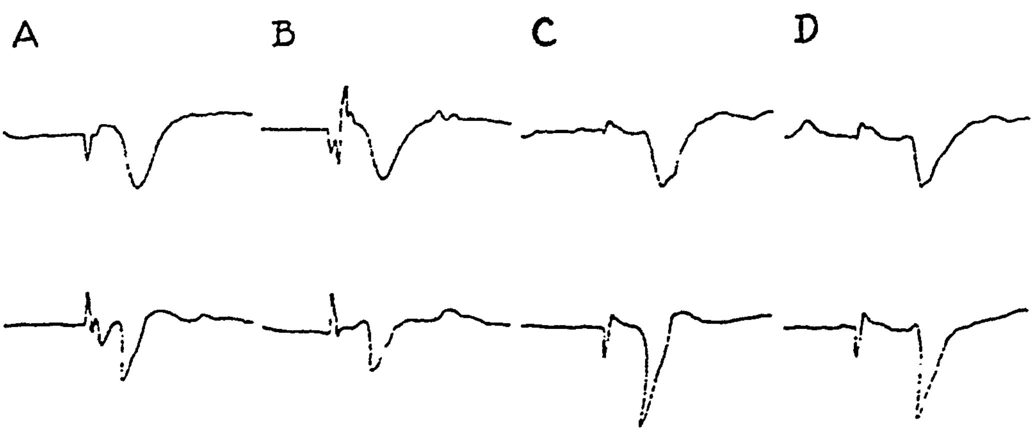

Fig. 4. Comparative latency of responses from different regions. Electrograms of left (upper record) and right posterior sigmoid gyri:

A. Left sciatic stimulated.

B. Right sciatic.

C. In region of Forel's field H, at level of mammillary bodies 5 mm. from midline. See figure 6 B.

D. Fornix (?) See text.

and 6). The localization of the response in the cortex is more or less sharp, depending upon the region stimulated. Stimulation of the substance of the thalamus tends to produce more discretely localized responses than does stimulation of the capsule or of the medial lemniscus. Repetition of the stimuli to form a series at a frequency of 1 to 4 per sec. occasionally led to an increase in the response suggesting a phenomenon akin to recruitment (fig. 2, C).

B. *The short-latency response followed by repetitive bursts of activity.* This response, like the preceding one, is most frequently seen in the sensorimotor cortex, and also is found only on the side of stimulation (fig. 3). It has been produced most frequently when the stimulating electrodes were placed in the anterior nucleus of the thalamus, the external medullary lamina, and the internal capsule.

The localization in the cortex and in the thalamus of the two types of response described above is admittedly incomplete at present. Indeed, the description given is undoubtedly an oversimplification, for the spike followed by bursts was on one occasion produced by stimulation of the ventral nucleus and stimulation of the anterior nucleus did not invariably

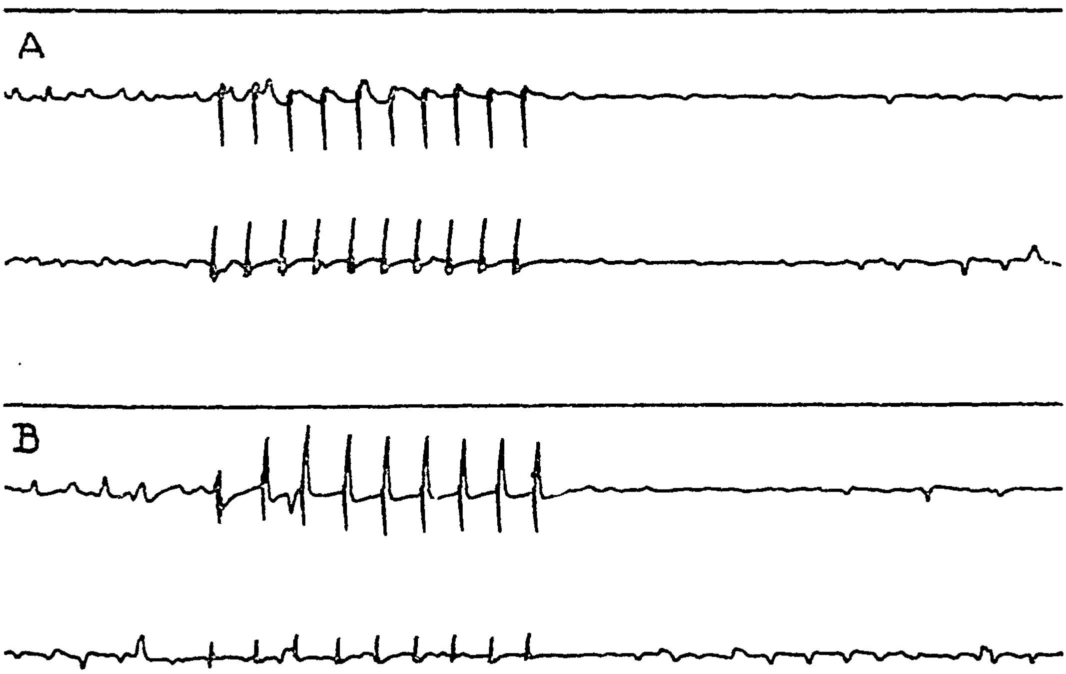

Fig. 5. Inhibition of spontaneous activity. Electrograms of left (upper record) and right posterior sigmoid gyri.

A. Stimuli to posterior part of head of left caudate.

B. Internal capsule slightly anterior to A.

produce the full response. The present results are included here because of their bearing upon the problem of the localization of the afferent pathways to the cortex which are involved in the production of primary and secondary responses after sciatic stimulation (Dempsey, Morison and Morison, 1941).

C. *The medium latency, generalized response.* Stimulation of the subthalamic regions is followed by a response in all parts of both cortices. The latency of this response varies from 30 to 80 msec. in different animals. In any animal the latency is identical or nearly identical with that of the secondary response to sciatic stimulation (fig. 4). The polarity, magnitude and duration of the response likewise are similar to these features of the

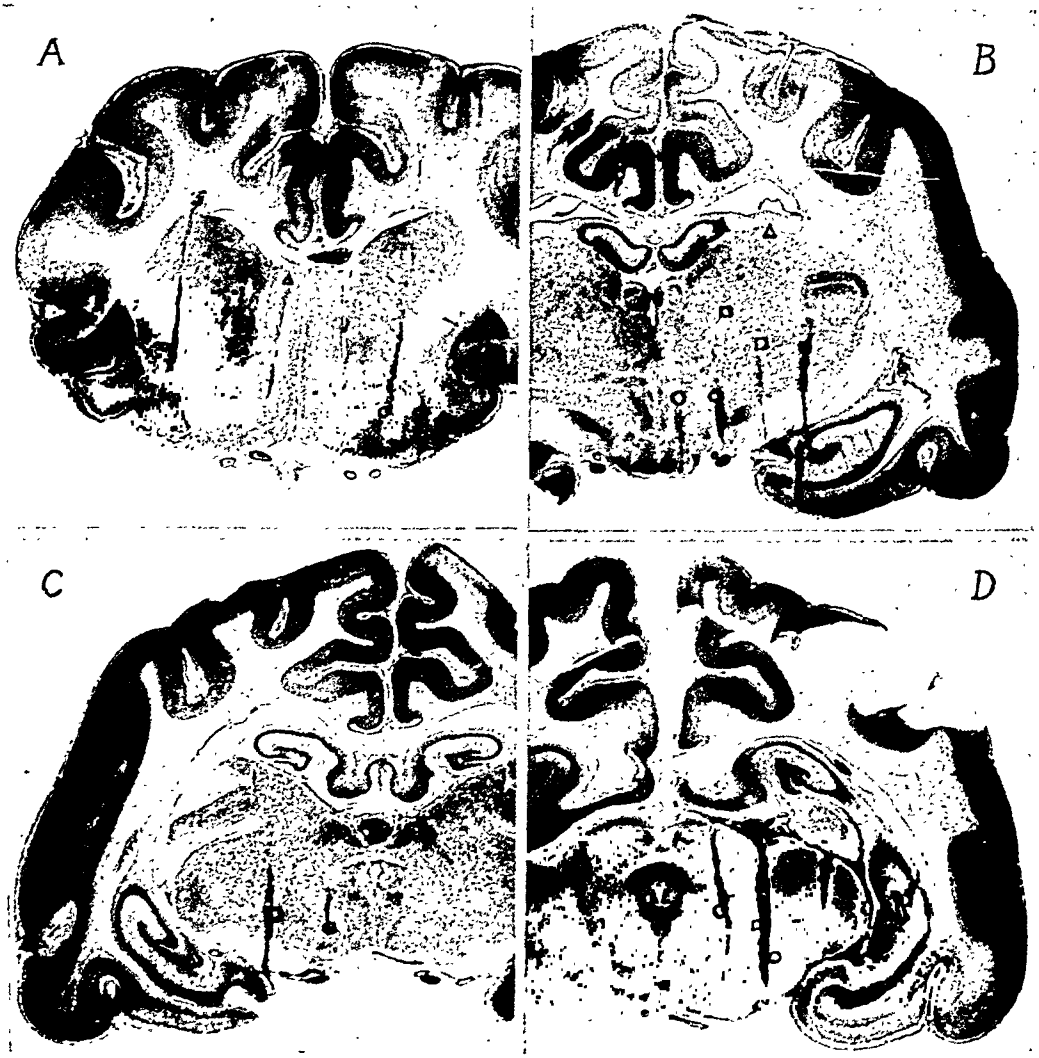

Fig. 6. Photomicrographs of Nissl sections of the cat's brain showing points from which responses similar to those illustrated in the accompanying figures were obtained. A, B, C from the same experiment; D, another preparation. Circles: Medium latency response (fig. 4 C). Triangles: Long latency response (fig. 4 D). Squares: Short latency response (fig. 2 A and B).

A. Section through the head of caudate, anterior part of anterior commissure and rostral pole of amygdala.

B. Section through habenular ganglia, and mammillary bodies.

C. Section through posterior commissure and habenulo-peduncular tract.

D. Section through large celled part of red nucleus. Stimulation of the points shown in this section frequently produced both short and medium latency responses, not illustrated but similar to 4 A and B.

secondary response. This generalized response has been obtained when the stimulating electrodes were placed in several points in the ventral part of the midbrain which contain bundles of fibers of the lemniscus system as they separate into medial and lateral components to enter

medial and lateral diencephalic areas (fig. 6, C and D). Slightly rostrally, responses were most consistently obtained in the region of the ventral part of the N. subparafascicularis, while at the level of the mammillary bodies active points were located in the region of the fields of H_1 and H_2 of Forel from 1 to 5 mm. from the midline, never more laterally and never so far dorsally as to include thalamic structures (fig. 6, B). Further rostrally, responses were less consistently obtained, but when they occurred the active areas were similarly located in the subthalamus.

The most rostral point which gave responses (4 out of 7 expts.) was situated at approximately the level of figure 6, A and was found to lie just ventral to the anterior part of the anterior commissure (5–7 mm. from the midline). Of the many nuclei and fiber tracts in this region, the medial part of the rostral pole of the amygdaloid complex was apparently most closely related to the stimulating electrodes.

D. *The long-latency, generalized response.* A response whose latency is 100 to 250 msec., and whose polarity and wave form are similar to those of the medium latency response, occurred after simulation of certain areas in the brain. This response is indistinguishable from the medium latency response and from the secondary response to sciatic stimulation, except for its latency (fig. 4, D). It has been obtained from the cortical grey and the associated white matter of the cingular gyrus, and also when the electrode tip, as judged by comparisons of the recording of the depth scale with the sectioned brain, was in the corpus callosum or the immediately subjoined fibers of the fornix system (fig. 6, A and B). Since this part of the brain is easily distorted by the pressure of the needle, and there is grave danger of spread of current in the ventricular fluid, it is difficult to be certain whether the fornix as well as the cortex was involved. Future stimulations under direct vision and with the ventricle emptied of fluid may help to elucidate this point.

E. *The inhibition of spontaneous activity.* In several experiments stimulation decreased the spontaneous activity of the cortex. This effect was produced best by a series of stimuli at a frequency of 1 to 4 per second, although it could be demonstrated occasionally after single shocks. Frequently there was an after-discharge of inhibition which outlasted the stimulation by several seconds (fig. 5). Inhibition was not tested routinely, since in the majority of the experiments only the responses to single shocks were observed. Nevertheless, inhibition has been seen when the stimulating electrodes were placed in the caudate nucleus (cf. Dusser de Barenne and McCulloch, 1938b, for the similar effect of strychninization of the same area), in the associated parts of the internal capsule, and even in the corona radiata above the corpus callosum. When the internal capsule was activated the inhibition was confined to the cortex on the side stimulated, while in the other experiments inhibition of activity in both cortices resulted.

Discussion. The experiments described in the preceding sections were undertaken because of their bearing upon the localization of the afferent pathways involved in the production of primary and secondary cortical discharges after sensory stimulation.

It has been shown elsewhere that these responses to sensory stimulation can be selectively abolished by properly placed lesions (Dempsey, Morison and Morison, 1941). The question arises, therefore, whether the results from stimulation of points in the brain can be homologized with the results obtained from stimulation of sensory nerves.

The primary response to sciatic stimulation has a short latency (8 to 10 msec.), is sharply localized in the leg sensorimotor area on the side opposite to the stimulus, and is abolished by lesions which involve the lateral part of the medial lemniscus or the ventrolateral nuclei of the thalamus (Dempsey, Morison and Morison, 1941). In the experiments presented here it is shown that stimulation of the ventrolateral thalamic nuclei, the lateral part of the medial lemniscus, or the thalamic radiations is followed by a fast response in the ipsilateral sensorimotor cortex. It appears, therefore, that the responses to contralateral sciatic stimulation and to ipsilateral thalamic stimulation may both be due to activation of the same cerebral pathway. It is hardly necessary to point out that this path coincides with the well-known course of somatic sensation as described by classical anatomy. Reference may also be made here to the discussion by Dempsey et al. (1941) of the similarity of this response to the "primary" response of Forbes and Morison and the cortical activation by touch stimulation of Marshall, Woolsey and Bard (1937).

It should be emphasized that in the present investigation no systematic study of the localization in the cortex of the representation of specific parts of the ventrolateral nuclear mass of the thalamus has been attempted. It seems probable, however, that the method employed will reveal discrete representation of face, leg, and arm areas in the thalamus of the cat as has been shown anatomically by a number of authors (cf. Walker, 1938) and physiologically (Dusser de Barenne and McCulloch, 1938a) for primates. The further possibility that the cortical representation of ventralis pars arcuata may differ from that of pars externa, as has been suggested by Walker for the homologous nuclei of primates, remains to be investigated.

There are also essential similarities between the secondary response to sciatic stimulation and the medium-latency generalized response. Both occur at the same latency and both have the same appearance with regard to polarity, voltage and duration. The secondary response is abolished by lesions which destroy the subthalamic regions, and the medium-latency response occurs after stimulation of these regions. It appears, therefore,

that here, too, we are dealing with activation of the same pathways after either sciatic or central stimulation.

Just what fibers form the pathway for the secondary response is difficult to determine. Presumably at midbrain and lower diencephalic levels they are represented by the medial divisions of the medial lemniscus which have a roughly similar course (cf. Rioch, 1931). Cajal (1911) indeed has followed collaterals into the zona incerta, a course exceedingly similar to that required by these experiments. Although good responses were obtained in the region of the thalamic fasciculus of Forel, it is unlikely that the dento-rubro-thalamic tract is involved since the presence of the dentate nuclei (Dempsey et al., 1941) is not essential for responses to sciatic stimulation, and stimulation of the terminal nucleus of the tract (ventralis pars arcuata (Ranson and Ingram, 1932)) does not produce the phenomenon. Indirect somatic afferent connections with the basal telencephalic grey situated in the region of our most rostral active points have been suggested by various authors but have no very well recognized status. For the present it may only be asserted that impulses traveling probably over the medial division of the medial lemniscus course through the dorsal and medial part of the subthalamus to the region of the anterior pole of the amygdala, and thence are widely distributed to the cortex (Morison, Dempsey and Morison, 1941). Judged from the very small difference in latency between responses elicited by stimulation anywhere along this path, conduction through the entire system from sciatic to basal telencephalon must be very rapid. This suggests that a minimum of internuncial neurons is involved.

The fast response without the medium-latency response occurs after stimulation of the ventrolateral nuclei of the thalamus (fig. 2), and the medium-latency effect is produced without the fast effect when stimuli are applied in the region of the fields of Forel (fig. 4) or rostral thereto. These two responses are therefore completely separable in stimulation experiments. Similarly, in lesion experiments selective abolition of the primary and secondary responses to sensory stimulation was produced (Dempsey, Morison and Morison, 1941). It appears abundantly clear, therefore, that the two components in the cortical response to sensory stimulation occur as a result of activation of two separate and distinct afferent pathways, at least beyond the level of the midbrain.

The long-latency generalized response appears to be identical with the medium-latency response in all respects except latency. Since the latency is longer than that observed after sensory stimulation, it is clear that the regions from which the slow response is elicited cannot represent parts of the afferent pathway from the sciatic. The similarities in the wave forms are so great, however, that they suggest that the same final response may

be produced by activation from different afferents. If this hypothesis be correct, the long latency should represent a slower conduction rate in its afferent pathway. It should be noted, however, that the thresholds for both long and medium latency responses are the same, and that these thresholds are also the same as those of mammalian A fibers.

SUMMARY

The brain stems of cats have been systematically explored with single stimuli applied through discrete electrodes, while recording cortical activity in 2 to 5 regions of the cerebral cortex. Five types of cortical response have been observed. These responses are as follows:

1. A response, which has a latency of 8 to 10 msec. and which is localized in the cortex of the side ipsilateral to stimulation, is produced by stimulation of the medial lemniscus, the ventrolateral nuclei of the thalamus, the thalamic radiations and the internal capsule (figs. 2 and 6). Reasons are given which indicate the identity of this response with the primary response to sensory stimulation (p. 740).

2. A fast response, which resembles that described above but which is followed by bursts of spikes similar to the spontaneous bursts of activity seen in the electrocorticogram from anesthetized animals, is produced by stimulation of the anterior nucleus of the thalamus, the thalamic radiations and the internal capsule (fig. 3).

3. A medium-latency (30 to 80 msec.) generalized response is produced in all regions of both cortices by stimulation in the region of the rostral pole of the amygdaloid complex, the dorsal part of the subthalamus, and the region of the nucleus subparafascicularis (figs. 4 and 6). This response is identical in wave form and nearly so in latency to the secondary response produced by sensory stimulation (p. 740).

4. A long-latency (100 to 250 msec.) generalized response which, except for latency, is identical with the medium-latency response, is produced by stimulation of the fornix, the corpus callosum, the radiations to the cingular and the suprasylvian gyri (figs. 4 C and 6). The possibility is discussed that this response represents activation of a slower afferent path to the response described in 3 above (p. 741).

5. Inhibition of spontaneous cortical activity may be produced by stimulation of the caudate nucleus, the internal capsule and the cortical radiations (fig. 5).

The bearing of these results on the localization of afferent pathways to the cortex from sensory stimulation is discussed (p. 740).

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ON THE PROPAGATION OF CERTAIN CORTICAL POTENTIALS

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Previous studies (cf. Dempsey, Morison and Morison, 1941) have shown that sensory stimulation gives rise, under certain conditions, to a widespread cortical discharge with a latency of from 30 to 80 msec. The presence of the thalamic nuclei is not necessary for the response, but subthalamic and apparently basal telencephalic mechanisms participate in its production. Further interest attaches to the phenomenon since its general form and distribution are entirely similar to those of the most prominent spontaneous cortical waves which occur either under deep barbiturate anesthesia or in animals in which the corticothalamic circuits responsible for the more usual cortical rhythms (Dusser de Barenne and McCulloch, 1938) have been interrupted. Forbes and Morison (1939) have shown that the response is no longer obtainable from the remaining parietal and occipital cortices after removal of all the tissue anterior to the level of the junction of the anterior and middle thirds of the lateral gyrus. They left unanswered, however, the question whether or not the frontal cortical substance itself is essential for the firing of the remaining cortex. As they recognized, their lesion might just as well have interrupted direct paths from basal regions which normally distribute the activity more or less simultaneously to all parts of the cortex. The principal question considered in this paper therefore is: does the widespread nature of this response reflect the activity of some definite cortical "trigger zone" spreading through short neuron chains of association to other cortical areas, or does it depend upon more or less direct paths from subcortical areas to all parts of the cortex irrespective of their connections with one another?

The problem may be conveniently separated into two divisions, one dealing with the spread of activity from one hemisphere to the other (the crossed response) and a second concerned with the transmission within one hemisphere.

Methods. The methods and apparatus used were similar to those described in accompanying papers (Dempsey et al., 1941; and Morison et al., 1941) and need not be discussed here.

Results. A. *Spread of activity from one hemisphere to the other, "the*

crossed response." In a previous paper (Dempsey, Morison and Morison, 1941), it was demonstrated that after hemisection or removal of various parts of the midbrain and diencephalon, sciatic stimulation still elicited a secondary discharge from both cortices. The activity in the cortex ipsilateral to the lesion, designated as the crossed response, was found to depend upon the integrity of the corpus callosum (loc. cit., p. 721).

Since the chief function of the latter structure is ordinarily believed to be the association of homologous points in the two cortices (cf. the trans-

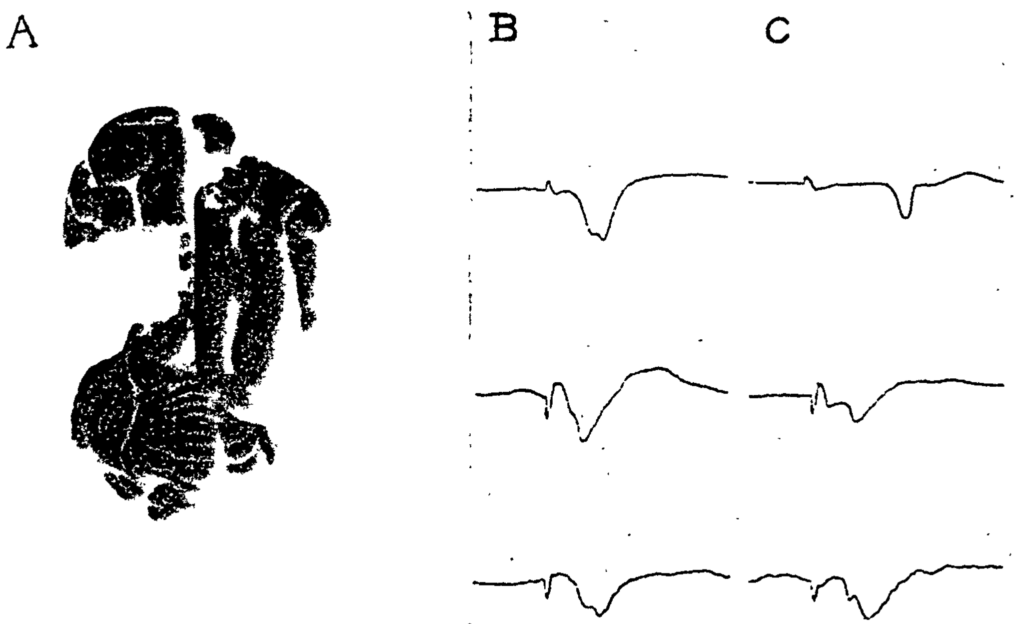

Fig. 1. A. Photograph of cat's brain after removal of cortical and basal areas on left, sparing the frontal cortex, and of the right anterior pole, sparing the fibers of the corpus callosum. Electrograms: records from above downwards: left anterior sigmoid gyrus; right anterior sigmoid gyrus; right posterior ectosylvian gyrus. Paper speed in this and succeeding records, 60 mm. per sec., unless otherwise indicated. B. After sciatic stimulation (at artifact), after production of the lesion on the left. C. Same, after removal of the right frontal pole. In this record the right posterior sigmoid gyrus electrodes were replaced on the edge of the lesion.

mission of strychnine spikes) (Gozzano, 1936) and of electrically induced activity (Curtis and Bard, 1940), the following experiment was done.

In two preparations in which all cortical and basal structures between the anterior margin of the thalamus and the colliculi had been removed on the left side, the right cortex anterior to this level was removed by a sloping cut sparing the anterior third of the callosum. Responses to sciatic stimulation were still present in all parts of the remaining cortex even though the homologous opposite portions were absent (fig. 1). Further evidence that the callosal fibers responsible for the crossed response form a rather definite bundle different from those connecting symmetrical points was

derived from the following type of experiment. The brain stem was hemisected at the intercollicular level and the ipsilateral cortex explored for responses after section of various parts of the callosum. Responses were found to be present over the entire cortex with as much as the posterior two-thirds of the callosum divided. Conversely, in other experiments, section of merely the anterior third wholly abolished the crossed response.

B. *Spread of activity within a single hemisphere.* The observation by Forbes and Morison (1939) that removal of all tissue frontal to a perpendicular plane at the posterior border of the anterior third of the lateral

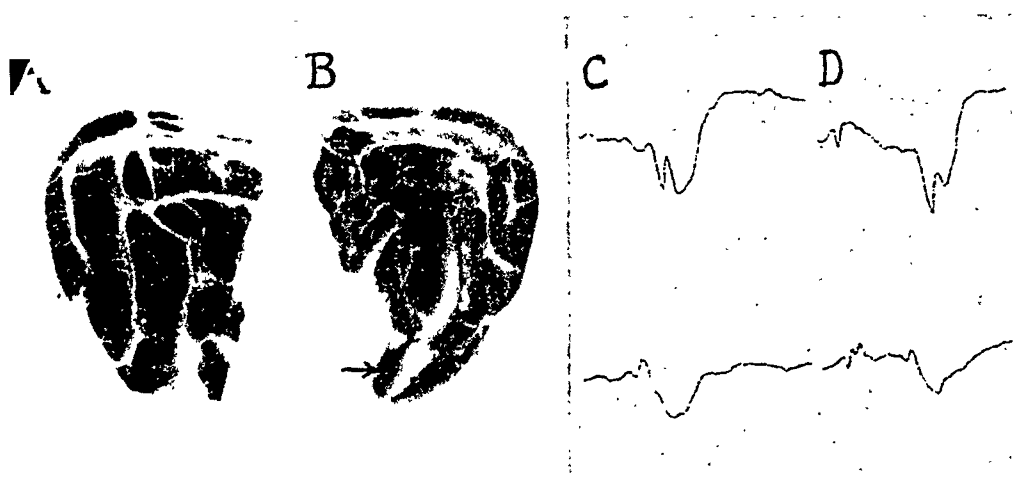

Fig. 2. Photographs of right cortical remnant deprived of all its connections with the rest of the brain except for the small bit of white matter, indicated by arrow, at the tip of the temporal horn of the ventricle. A. Lateral view. B. Medial view. C. Electrogram: upper line, left posterior ectosylvian gyrus; lower line, right conjugate cortex. The spontaneous activity from the cortex represented in 2 A and B is synchronous with that in the opposite normal cortex. D. Electrogram: secondary responses to sciatic stimulation in the normal left cortex (upper line) and right occipital cortex shown in A and B (lower line) are shown.

gyrus abolished the response in the remaining cortex was confirmed. Similarly, in preparations with midbrain hemisections, the preceding operation, though performed only on the contralateral side, abolished the response in all the remaining cortex. It is important to point out, however, that removal of similar amounts of neocortex without damage to basal structures did not affect the response in any remaining cortex (cf. fig. 1). In fact, the response was still present over the entire cortical area illustrated in figure 2 after severance of all its connections with the rest of the brain save for a small group of fibers in the region of the temporal horn of the ventricle (fig. 2, B). These experiments constitute strong evidence that the response in the occipital and parietal cortex does not depend upon the presence of the more frontally situated cortical areas

and is therefore not cortico-cortical. On the other hand, a relatively localized stab wound in the region spared by the preceding type of operation abolished the response over a wide area of the homolateral occipital cortex (figs. 3, A and C; 4, A and B). It might be objected that the disappearance of the response in the latter type of experiment was due to interference with the blood supply or to other nonspecific factors. Such an interpretation is inadmissible. Great care was taken to avoid severing major arteries when making the operation, and the cortical vessels remained well filled after the procedure which was indeed much less extreme than many other operations without effect on the responses. Furthermore, the application of strychnine to a small part of either cortex resulted in typical strychnine "spikes" which were transmitted to the opposite conjugate part of the cortex (fig. 3, D and E). Not only did this establish the fact that the operated cortex was not seriously injured, but it emphasized that the secondary discharge to sciatic stimulation involves cortical elements which are different from those involved in the strychnine spikes, since only the latter were capable of setting up a contralateral response in these conditions.

The discovery that the impulses responsible for the secondary discharge travel up from the brain stem and spread over apparently direct paths to the cortex from an area in the region of the temporal horn of the ventricle raised a further question concerning the crossed response (see section A). Do the fibers which carry the response across the callosum run directly to the various cortical areas, or do they first run down to the opposite temporal horn and make connection with the distributing system which arises there? The following experiment was therefore undertaken. The mid-brain was hemisected as in the previous experiment (p. 746) and a stab wound made on the *same* side far lateral and deep in the temporal lobe similar to that made contralaterally in other preparations. This lesion prevented the response of a corresponding part of the ipsilateral occipital cortex. Another lesion anteriorly placed but still far lateral in the cortex abolished the response in the frontal cortex. Subsequent unilateral application of strychnine to small cortical areas demonstrated the intactness of the known callosal cortical association fibers (fig. 3, E). The conclusion seems unescapable that the crossed response does not reach the opposite cortex directly through cortico-cortical association bundles, but first makes connection with structures deep in the temporal lobe and is thence distributed to the cortex of that side.

C. *Effects of the foregoing lesions on a component of the spontaneous cortical activity.* It has previously been pointed out (Forbes and Morison, 1939) that under deep nembutal anesthesia the spontaneous cortical activity may be almost completely reduced to a series of widely separated waves each of which bears a striking resemblance to the secondary dis-

charge elicited by sciatic stimulation. The simultaneous recording of various widely separated cortical areas in the present study showed that this activity occurred at nearly the same time throughout the cortex as long as the conducting systems outlined in sections A and B of this paper remained intact. Slight differences in the time of occurrence of the ac-

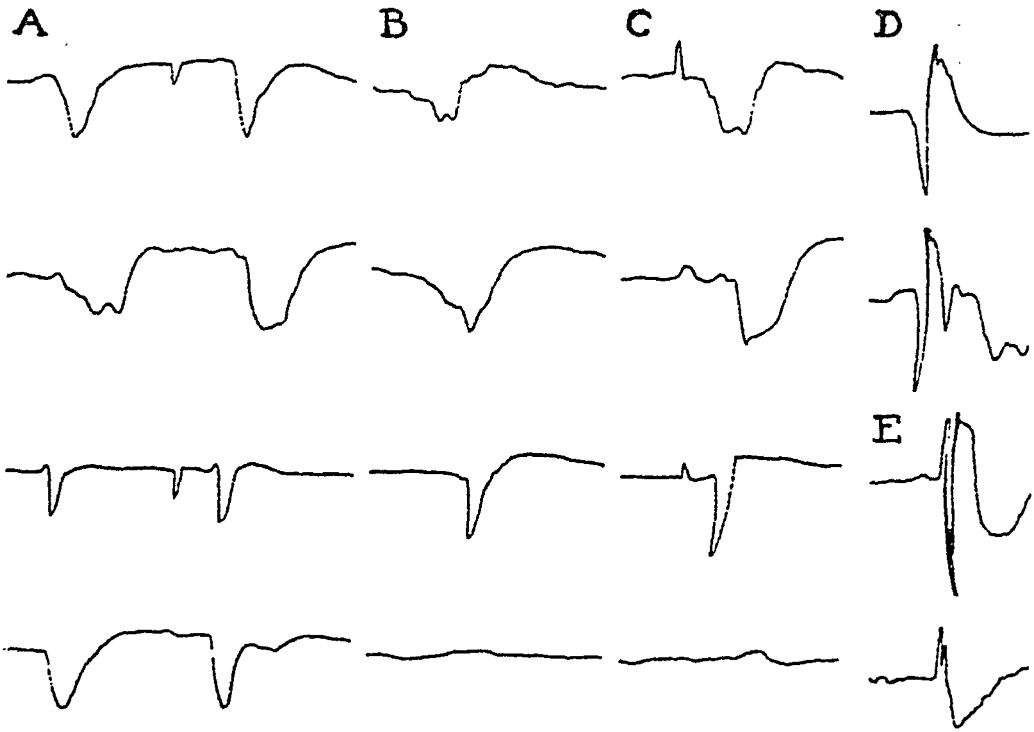

Fig. 3. Dependence of certain types of activity upon the integrity of fiber system arising near temporal horn of ventricle. See text. Electrograms from above downwards: left anterior sigmoid gyrus; left posterior ectosylvian gyrus; right anterior sigmoid gyrus; and right posterior ectosylvian gyrus. A. Spontaneous activity and secondary response to sciatic stimulation. B. Spontaneous activity after the lesion illustrated in figure 4 had been produced. C. Secondary response to sciatic stimulation after the lesion shown in figure 4. D. Strychnine "spike" induced by application of strychnine on the left posterior ectosylvian gyrus (lower record) and transmitted to the normal opposite conjugate cortex (upper record). E. Another preparation. Strychnine "spike" transmitted from right posterior ectosylvian gyrus (upper record) to opposite conjugate cortex after complete abolition of secondary responses and spontaneous activity by a lesion in the region of the right temporal horn of the ventricle.

tivity in various parts of the cortex of one hemisphere and somewhat longer intervals between its appearance on the two sides (either side might lead) are apparently explicable as the time necessary for conduction, but within these limits the activity may be referred to as synchronous throughout the cortex. Especially noteworthy is the fact that this type of activity

was particularly striking in preparations in which extensive damage had been done to the thalamus either acutely or some months previously (fig. 5, A).

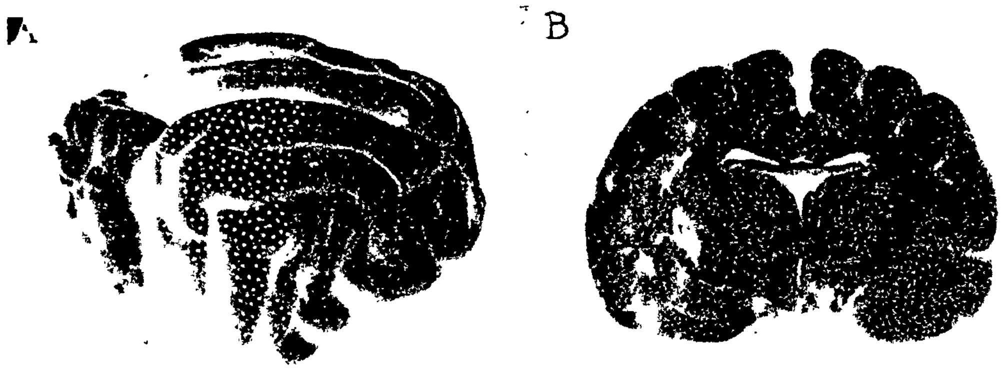

Fig. 4. Photographs of cat's brain from which the responses shown in figure 3 were obtained. A. Lateral view, indicating location of lesion. The stippled portion represents the area from which responses were abolished. B. Cross section, indicating extent of lesion.

Fig. 5. Electrogram from an animal in which the left thalamus had been removed three weeks previously. Upper record, left posterior sigmoid gyrus; lower record, right posterior sigmoid gyrus. Paper speed, 15 mm. per sec. A. Record showing synchronization of spontaneous activity in the two hemispheres. B. Lack of synchronization after complete section of the corpus callosum.

A further more detailed analysis of these preparations is in progress, but as Dusser de Barenne and McCulloch (1938) have shown, interruption of thalamocortical circuits leads to permanent abnormalities of the cortical activity, especially noticeable as a suppression of the predominant cortical rhythm. The rhythmically occurring but widely separated waves may, on the other hand, actually be enhanced.

Throughout the course of the work tracing the cortical spread of the secondary discharge to sensory stimulation, it was noticed that lesions which abolished the latter also affected the spontaneously occurring waves of similar appearance. Callosal lesions which prevented the crossed secondary discharge dissociated the rhythmic activity of the two sides although spontaneous activity continued synchronously within each hemisphere. Temporal lobe lesions abolishing the secondary discharge in a part of either hemisphere also abolished the spontaneous waves of the same type. Midbrain lesions which abolished the secondary discharge (Dempsey et al., 1940) did not depress but in some preparations actually enhanced the spontaneous waves.

Discussion. It appears from the foregoing experiments that the secondary cortical discharge elicited by sciatic stimulation reaches the cortex over specific paths which arise deep in the temporal lobe in the region of the temporal horn of the ventricle and thence spread widely over the homolateral cortex. Furthermore, this region is in connection, via the anterior part of the callosum, with the corresponding contralateral area and is capable of activating through it the entire contralateral cortex. The spontaneous cortical rhythm made up of widely separated large waves similar in appearance to the secondary discharge may also be said to arise in this temporal area and to be synchronized throughout the cortex by means of the conducting system outlined for the secondary discharge.

It is impossible at present to give a specific localization to the active temporal area and even less satisfying to attempt a correlation of the present findings with what is known of the anatomy of these regions. Stimulation experiments (Morison, Dempsey and Morison, 1941) have shown that the response can be elicited from a region in which the most significant structure is the rostral pole of the amygdala. Several other basal telencephalic nuclei must be considered, however, and several notable fiber systems, of which the medial forebrain bundle, the stria terminalis, and the external capsule are the most prominent, might also have been involved in both the stimulation experiments and the lesions reported here. The external capsule would seem to offer the advantage of having the most generally admitted connections to the cortex, but evidence in the literature for extrathalamic somatic afferent connections for any of these areas is at best vague and unsatisfactory.

Another provocative finding is the connection of the two active basal

areas via a small portion of the callosum. It would certainly have been more in accord with known anatomy to find that the anterior commissure was responsible, but crossed responses were seen in several preparations in which it was completely divided. The relation of the corpus callosum to the older commissural systems has never been completely clear, however, and, of course, as old a structure as the hippocampus is intimately bound to the newer system. Could it not be therefore that some amygdala-amygdaloid or other baso-basal connections may have left the anterior commissure to travel with the callosum? It is hard to interpret the present results on any other basis.

It is difficult to suggest a correlation between this striking type of electrical activity of the cortex and the function of the cortex as it affects animal behavior. Secondary discharges similar to those under discussion have been elicited by various types of afferent stimulation, optic (Bishop and O'Leary, 1936) and even labyrinthine (Gerebetzoff, 1940). The latter author makes the intriguing suggestion that his responses may represent the generalized waking up of the cortex as the animal falls off a branch on which he is asleep. The notion has a good deal to recommend it and applies equally well to other sense modalities. In the present state of our knowledge, however, any behavioral correlates to cerebral action potential patterns are bound to be tentative.

SUMMARY

The presence in the cortex of a secondary response to sciatic stimulation homolateral to a hemisection of the brain stem was found to depend upon the presence of the anterior third of the corpus callosum. The opposite conjugate region of the cortex was not, however, essential (fig. 1).

Evidence is presented which indicates that in each hemisphere the response is distributed to all parts of the cortex by a fiber system which originates in an area close to the temporal horn of the ventricle (figs. 2 and 4). Other known cortico-cortical association systems are apparently incapable of spreading the response.

A type of spontaneous cortical rhythm is described which is dependent upon the same conducting system and not upon corticothalamic circuits (figs. 3 and 5).

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THE EFFECT OF DESICCATED HOG BILE AND HOG BILE ACID PREPARATIONS ON THE VOLUME AND CONSTITUENTS OF BILE[1]

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The essential bile acid in hog bile is hyodesoxycholic acid, or 3-6 dihydroxycholanic acid. It is not found in human bile and differs from desoxycholic acid in that it has a hydroxyl group on carbon 6 instead of on carbon 7. The accepted chemical formula for hyodesoxycholic acid has been given in a previous publication (1). Recently, Fernholz (2) isolated from hog bile a keto acid whose formula was 3-hydroxy-6-ketocholanic acid. This was later confirmed by Schoenheimer et al. (3). According to Irvin, Mecker, Anderson and Johnston (4) hyodesoxycholic acid represents 88 per cent of the total bile acids found in dried fresh hog gall bladder bile; the remainder consists of 3-hydroxy-6-ketocholanic acid. These investigators also reported that 94 per cent of the hog bile acids are combined with glycine and approximately 6 per cent are combined with taurine.

Irwin, Johnston and Anderson (5) have administered desiccated hog bile orally to biliary fistula dogs and reported that it increased the volume output of bile and the total output of bile acids. The greatest increase occurred in the "desoxycholic acid fraction," which was obtained by the difference between the cholic acid and total bile acid output. No data were provided in their abstract and no comparison of desiccated hog bile with ox bile was mentioned.

Since we (1) have been interested in ascertaining the comparative response of the dog's liver to ox-bile salts and oxidized cholic acid salts, we have extended our studies to include hog bile salts.

METHODS. In this study the same methods[2] were used as those outlined in our previous paper (1).

Two preparations of desiccated hog bile were used, one a "purified"

[1] This work was aided by the E. L. Dawes and Marjorie Newman Research Grants.

[2] The carbonyl assays were made at the Wilson Laboratories, Chicago, by a method devised by Dr. E. L. Gustus of the Wilson Laboratories, Chicago.

preparation obtained from Wilson Laboratories, Chicago, and the other "Desicol" as marketed by Parke, Davis and Company. The Wilson product consisted almost entirely of alpha-glycohyodesoxycholic acid and contained 0 to 2 per cent carbonyl groups. Desicol contained 3.3 per cent carbonyl groups.

Oxidized desiccated hog bile was also used and contained 5 per cent carbonyl groups. In addition three different preparations of hyodesoxycholic acid were used. One was pure unconjugated hyodesoxycholic acid,

TABLE 1

Effect of hog bile salt preparations on the volume and constituents of bile

REGIME	NO. OF DOGS	NO. OF TESTS	VOLUME, CC./24 HRS.			CHOLIC ACID, MGM./24 HRS.			CHOLESTEROL, MGM./24 HRS.			PIGMENT, MGM./24 HRS.			TOTAL SOLIDS, MGM./24 HRS.		
			C*	T†	Per cent chg.	C	T	Per cent chg.	C	T	Per cent chg.	C	T	Per cent chg.	C	T	Per cent chg.
3 grams desiccated hog bile	5	10	176	242	+37	1442	1490	+3	13	13	0	177	160	−9	29	30	+3
3 grams Desicol	5	10	165	230	+39	1400	1510	+7	12	13	+8	171	173	+1	29	32	+10
3 grams oxidized desiccated hog bile	6	7	123	173	+40	1339	1280	−4	10	12	+20	89	82	−8			
3 grams unconjugated unoxidized hyodesoxycholic acid	5	8	171	237	+38	1394	1439	+3	16	14	−12	207	185	−10	29	29	0
3 grams oxidized unconjugated hyodesoxycholic acid	5	5	157	209	+33	1382	1365	−1	9	11	+22	108	117	+8	29	31	+7
3 grams oxidized conjugated hyodesoxycholic acid	5	6	153	198	+30	1440	1427	0	11	14	+27	195	168	−13	31	32	+3
3 grams ox-bile salts; 1.71 grams cholic acid	8	15	133	182	+36	1493	2809	+88	12	16	+33	115	105	−8	36	44	+22
3 grams oxidized conjugated ox-bile salts	11	14	126	175	+39	1435	1559	+8	11	15	+36	104	100	−4	39	40	+2
3 grams Decholin dehydrocholic or oxidized cholic acid	6	10	128	264	+106	1473	1647	+12	11	8	−27	114	129	+13	38	30	−22
3 grams Ketochol or Kebilac, Ketocholanic acids	7	10	126	251	+99	1468	1568	+6	11	14	+27	114	105	−7	35	32	−9

* "C" = control.
† "T" = treated.

which contained 0 to 3 per cent carbonyl groups. Another was the oxidized unconjugated hyodesoxycholic acid, which contained 15.4 per cent carbonyl groups. The other was oxidized conjugated hyodesoxycholic acid, which contained 8.1 per cent carbonyl groups.

By studying these various types of desiccated hog bile and hog bile acids we should be able to ascertain the comparative effects of hog bile products on the secretion of bile and its constituents. Furthermore, since we believe that the hydrocholeresis produced by a bile acid may be asso-

ciated with the position and number of hydroxyl groups and also with the effect of oxidation and conjugation, a study of these 3,6-dihydroxycholanic derivatives should yield a greater knowledge of the possible mechanisms and causes of hydrocholeresis.

RESULTS. *Volume output of bile.* (Table 1.) Three grams of the two preparations of *desiccated hog bile* increased the volume output of bile by approximately 38 per cent. This amount of choleresis is very similar (36 per cent) to that obtained with 3 grams of "natural" ox-bile salts. That is, ox-bile salts and desiccated hog bile per gram weight are equal in increasing the volume output of bile.

Three grams of *oxidized desiccated hog bile,* which contained 5 per cent carbonyl groups and consisted almost entirely of oxidized glycohyodesoxycholic acid increased the volume output 40 per cent. This amount of choleresis is very similar (39 per cent) to that obtained with 3 grams of oxidized conjugated ox-bile acids. Thus, oxidation of the *conjugated* hog or ox-bile salts does not increase the choleretic action of the "natural" bile acids in hog or ox bile.

Three grams of *pure hyodesoxycholic acid* increased the volume output of bile by 38 per cent, which is the same as that obtained with desiccated hog bile and also oxidized conjugated hog bile, and also ox bile salts and oxidized conjugated ox bile salts (table 1).

Three grams of *oxidized hyodesoxycholic acid* increased the volume output by 33 per cent. This is most interesting since oxidation of cholic acid markedly increases its choleretic property, which is not true of hyodesoxycholic acid.

Three grams of *oxidized conjugated hyodesoxycholic acid* increased the volume output by 30 per cent, which is not quite as large an increase (40 per cent) as obtained with oxidized desiccated hog bile.

Comment. Thus, oxidation or conjugation of hyodesoxycholic acid does not significantly modify its choleretic property.

Cholic acid output. The normal variation in daily cholic acid output in these Rous-McMaster biliary fistula dogs was ±10 per cent. Using this as our standard it can be seen from table 1 (cholic acid column) that desiccated hog bile and the various hog bile acid preparations did not change the synthesis or output of cholic acid. However, we have noticed that, when 3 grams of the hog bile preparations were administered, significant variations in cholic acid output occurred in the individual tests, the data for which are too bulky to present. For example, when 3 grams of the desiccated hog bile (Wilson) were given, in one case there was a decrease in cholic acid output of 19 per cent, and in another case an increase of 20 per cent. Variations in cholic acid output occurred with all the hog bile preparations that we used, but in all cases the decreases were relatively small and were counter-balanced by increases so that the average results

showed no appreciable change in cholic acid synthesis. In a previous report (1) we observed that occasional significant depressions in cholic acid output occurred when ketocholanic acids prepared from ox bile were administered, but only when large doses such as 5 grams were fed daily.

TABLE 2

Effect of hog bile salt preparations on the output of carbonyl groups in bile

BILE ACID	CC. PER DAY	MGM. TOTAL CHOLATES PER DAY	CONC. CHOLATES PER DAY	MGM. TOTAL KETO GROUPS PER DAY	CONC. KETO GROUPS PER CC.	TOTAL INCREASE DUE TO KETO ACID FED	MGM. EXCRETED OF KETO ACID FED	PER CENT RECOVERY OF KETO ACID FED
Desiccated hog bile (Wilson) 3 grams. 0-3% C=O group:								
Control	176	1442	8.1	53.0	0.30	0		
Treated	242	1490	6.1	52.8	0.21	0	0	
Desicol 3 grams. 3.3% C=O:								
Control	165	1400	8.4	53.0	0.32	0		
Treated	230	1510	6.5	42.9	0.18	−10.1	0	
Hyodesoxycholic acid 3 grams. 0-3% C=O group:								
Control	171	1394	8.1	53.0	0.31	0		
Treated	237	1439	6.0	37.6	0.15	−15.4	0	
Oxidized unconj. hyodesoxycholic 3 grams. 15.4% C=O:								
Control	157	1382	8.7	53.0	0.33	0		
Treated	209	1365	6.5	93.2	0.35	40.2	260	8.7
Oxidized conj. hyodesoxycholic 3 grams. 8.1% C=O:								
Control	153	1440	9.4	53.0	0.34	0		
Treated	198	1427	7.2	71.9	0.35	18.9	233	7.7
Oxidized hog bile 3 grams. 5.0% C=O:								
Control	123	1339	10.8	53.0	0.43	0		
Treated	173	1280	7.4	47.0	0.27	−6.0	0	

Output and recovery of carbonyl groups. Very little change occurred in the excretion of carbonyl groups when desiccated hog bile or hog bile acid preparations were administered orally (table 2). The total control output of carbonyl groups per day in this series of biliary fistula dogs was

53.0 mgm. Desiccated hog bile which only contained a small amount of carbonyl groups had no effect on the output of carbonyl groups. Desicol and pure hyodesoxycholic acid decreased the total output of carbonyl groups as well as the concentration per cubic centimeter. The latter decrease is only slightly significant. Both of these preparations contain approximately 3.0 per cent carbonyl groups. When oxidized conjugated hyodesoxycholic acid, containing 8.1 per cent carbonyl groups, was administered orally the carbonyl output was increased from 53 mgm. to 71.9 mgm. daily. This represented 233 mgm. of oxidized conjugated hyodesoxycholic acid excreted, or a recovery of approximately 8 per cent of the bile acid administered (1). In the calculation of the recovery of carbonyl groups, method III described in a previous paper was used (1). Oxidized unconjugated hyodesoxycholic acid which contains 15.4 per cent carbonyl groups increased the carbonyl output from 53.0 mgm. to 93.2 mgm. daily. The greater output of carbonyl groups is directly related to the greater amount of carbonyl groups present in this preparation. Approximately 260 mgm. of the oxidized unconjugated hyodesoxycholic acid was excreted which represents only a 9 per cent recovery. When the oxidized desiccated hog bile was administered, which contained 5.0 per cent carbonyl groups, there was no significant change in carbonyl output.

It can be seen that the oxidized hog bile preparations used did not greatly affect the carbonyl output in the bile. Approximately 8 per cent of the oxidized bile acids fed was recovered in the bile. This differs quantitatively from our observations on the metabolism of the oxidized ox-bile salts (1). When the oxidized natural bile acids were administered in 3 gram daily doses, from 16 per cent to 29 per cent was recovered during the actual period of administration. There was also a direct relationship between the amount of oxidized bile acid recovered in the bile and the percentage of carbonyl groups in the bile salt preparation that was administered. For instance, Dechacid no.14 contained 11.2 per cent carbonyl groups and the amount recovered was approximately 16.0 per cent (1). Ketochols which contained 18.3 per cent carbonyl groups, showed a 24.0 per cent recovery, while Decholin, containing 20.9 per cent carbonyl groups had a 28 per cent recovery. The oxidized hog bile acid preparations showed no such relationship. Although the percentage of carbonyl groups in the oxidized unconjugated hyodesoxycholic acid was twice as great as in the oxidized conjugated hyodesoxycholic acid, the amount recovered in either case was practically the same, approximately 8 per cent. What happened to the bulk of the hog bile acids administered is unknown. To be converted into cholic acid and excreted as such would require the addition of another hydroxyl group on carbon 12 and the transferring of the hydroxyl on C_6 to C_7. Such a transformation did not seem to occur since the cholic acid output in all cases was not increased over the control

output. The oxidized hyodesoxycholic acid preparations could have been reduced to the 3-hydroxy-6 ketocholanic acid or to hyodesoxycholic acid and excreted as such. Because of the lack of adequate quantitative methods for the determination of these hog bile acids in dog bile, further information as to the fate and recovery of these hog bile acids was not obtained. As indicated by the observations of Irvin et al. (5), the hyodesoxycholic acid probably was excreted in the undetermined bile acid fraction.

Effect of cholesterol output. All of the hog bile acid preparations except the unoxidized unconjugated hyodesoxycholic acid caused an increase in cholesterol output. However, in no case was the increase physiologically significant although 3 grams of oxidized conjugated hyodesoxycholic acid gave a significant statistical increase in cholesterol output. When the pure hyodesoxycholic acid was administered in 3 gram daily doses there occurred a 12 per cent decrease in cholesterol output. This result is not significant, physiologically or statistically. None of the eight tests on five dogs showed a marked decrease in cholesterol output as we observed when 3 and 5 grams of Decholin were administered (1).

Effect on pigment output and total solids. The hog bile salt preparations studied had no significant effect on pigment output. Thus, neither the hog bile acids nor any of the bile acids studied by us, whether oxidized or unoxidized, materially affected the output of bile pigment.

None of the hog bile acids studied affected the concentration of total solids significantly. From the standpoint of non-volatile solids, the desiccated hog bile and the hog bile acid preparations did not "thicken" the bile as do the "natural" conjugated ox-bile salts, nor "thin" the bile as do the oxidized ox-bile salts (Decholin, Ketochol and Kebilac).

Discussion. This work represents an extension of our studies on bile acid metabolism in which we desire to ascertain the effect of various bile acids on the secretion of bile, the relationship of the structure of the bile acid to its choleretic effect, and finally, to study the action and the metabolism of the oxidized bile acids. In a previous study (1) we found that the oxidized unconjugated acids, such as oxidized cholic acid (Decholin) and the mixture of oxidized cholic, desoxycholic and lithocholic acids (Ketochol and Kebilac) were the most potent choloretics per gram weight. These preparations consist primarily of the tri-ketocholanic acid or oxidized cholic acid with the carbonyl groups on C_3, C_7, and C_{12}. It is interesting that hyodesoxycholic acid with the hydroxy groups on C_3 and C_6, whether it is conjugated or not, yields the same amount of choleresis as unoxidized conjugated and oxidized conjugated ox-bile acids. Since the oxidation of cholic acid increases its choleretic property and since the oxidation of conjugated cholic acid does not increase its choleretic property, conjugation might be presumed to decrease the choleretic effect of oxidation.

This may be true of cholic acid, but it certainly is not true for hyodesoxycholic acid, since oxidation of hyodesoxycholic acid or of its conjugated derivative glycohyodesoxycholic acid does not augment its choleretic action. It appears that the position of the carbonyl group or groups in the cholane nucleus is important in determining the choleretic property.

The desiccated hog bile and the hog bile acid preparations have no appreciable effects on the constituents of bile such as cholic acid, cholesterol, pigment and total solids. Hyodesoxycholic, which is the essential bile acid in these preparations, seems to be handled differently by the liver than any of the other bile acid preparations that we have studied. Relatively little of the carbonyl groups administered in these bile salts can be recovered in the bile. It is quite possible that the liver has changed the oxidized hyodesoxycholic acid to such an extent that our present chemical methods will not detect it in the bile. Adequate quantitative chemical methods are needed for the detection and the determination of hyodesoxycholic acid and its related keto acid before a complete knowledge of the metabolism of hyodesoxycholic acid can be elucidated.

Thus, when hog bile preparations are used in man, as judged from our results on dogs, they will produce an increase in bile volume output equivalent to that obtained with equivalent amounts of unoxidized conjugated ox-bile salts or "natural" ox-bile salts. They will not in equivalent amounts cause as much choleresis as oxidized unconjugated cholic acid (ox-bile). The hog bile preparations do not "thin" the bile like oxidized unconjugated cholic acid (Decholin, Ketochol and Kebilac). When hog bile preparations are used, hyodesoxycholic acid, a bile acid apparently foreign to human bile is excreted into the intestine. This is not true when ox bile salts or oxidized cholic acid preparations are used. Whether this difference is of physiologic significance is unknown and moot.

SUMMARY AND CONCLUSIONS

1. Three grams daily of desiccated hog bile caused a 38 per cent (average) increase in the output of bile in biliary fistula dogs. This is the same as the increase obtained with 3 grams of ox-bile salts containing from 1.5 to 1.7 grams of cholic acid.

2. Three grams of pure hyodesoxycholic acid, the chief bile acid of hog bile, increased the output of bile to the same extent (40 per cent) as the desiccated hog bile.

3. Oxidation of desiccated hog bile or of the pure hyodesoxycholic acid did not augment the choleretic property of these bile acids. This is in contrast to the results obtained with cholic acid, the chief bile acid in ox bile.

4. Conjugation of oxidized hyodesoxycholic acid had no effect on the choleretic property. All the preparations of hog bile had about the same choleretic activity.

5. Whether oxidation of a bile acid augments its choleretic activity depends on the position of the carbonyl groups, and whether the conjugation of an oxidized bile acid decreases its choleretic activity depends on the position of the carbonyl groups.

6. The doses of the hog bile preparations used did not significantly modify cholic acid output, though the output of cholic acid was rendered more variable.

7. The hog bile preparations tended to increase total cholesterol output slightly, but had no significant effect on the total output of pigment and the concentration of non-volatile solids. The bile is not "thinned" by hog bile preparations as it is with oxidized unconjugated cholic acid preparations made from ox bile.

8. The recovery of material containing carbonyl groups in the bile is even less when oxidized hyodesoxycholic acid is given than when oxidized cholic acid is given (1).

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THE RELATION BETWEEN ELECTRICAL AND MECHANICAL EVENTS IN THE DOG'S HEART[1]

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In a preceding publication (1) concerned with the relations between electrical and mechanical events in the turtle's heart, the following facts were established: *a*. The onset of local contraction of surface muscle of the ventricles is coincident or nearly coincident in all regions, with the main peak of the differential potential-time curve derived from the region. *b*. Peaks on the differential curve occur during gradients on the unipolar potential-time curve and are proportional to the size of the gradient. The present communication is concerned with an extension of this study to the dog's heart.

METHODS. Myograms were recorded from local surface regions of the ventricles by means of an electrical resistance myograph, similar to the one used in the studies on the turtle (1). Local shortening of muscle segments results in an increase in the flow of electrical current through the myograph and the changes in current flow are amplified and recorded by a cathode ray oscilloscope. In one series of experiments myograms were recorded simultaneously with differential potential-time curves from the same region. To record the latter, the two arms of the myograph were provided with wick electrodes leading to a second amplifier and oscilloscope. In another series of experiments, the relations between myograph and pressure curves from the two ventricles and from the pulmonary artery were studied by recording the two curves simultaneously. For recording intraventricular or arterial pressures, a membrane manometer with photo-electric recording was used as in the previously reported experiments on the turtle's heart (2). In a third series of experiments, differential and unipolar potential-time curves were recorded simultaneously from various regions on the surface of the ventricles and the right auricle. Two zinc zinc-sulphate electrodes provided with a common wick, were mounted close together. The middle of the wick made contact with a small area of the cardiac surface and was held in place by a lightly stretched thread. Leads from the two electrodes to one amplifier and oscilloscope provided for the differential recording. Leads from one of the electrodes

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and from a distal electrode on a hind leg to a second amplifier and oscilloscope provided for the unipolar recording.

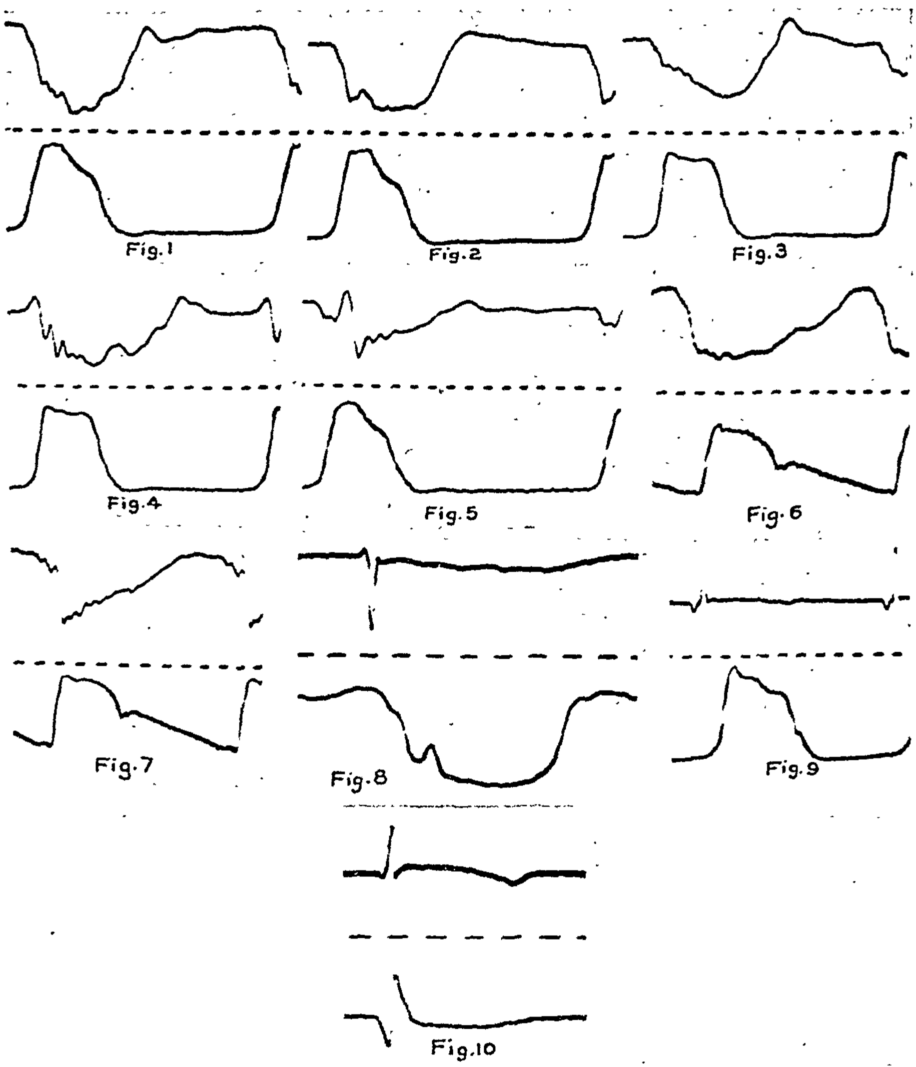
All experiments were carried out on large or medium sized dogs under morphine-ether anesthesia. The hearts were exposed in situ with open pericardium. The work was done in a laboratory surrounded by a double shield of wire netting to avoid interference from electrical strays. Measurements of all records were made with a micrometer comparator with 40X magnification.

RESULTS. *The relation of myograph and pressure curves.* In previous work on the turtle's ventricle (2), a clear relation was found to exist at all surface points between the onset of local contraction and the rise of intraventricular pressure. The intraventricular pressure curve shows an initial slow rise of pressure of a few millimeters of mercury, lasting about 0.12 sec. and terminating in a sharp increase in the pressure gradient. It was found that the onset of contraction of all surface points occurred during this initial rise of pressure or followed it by a brief interval. The occurrence of actual shortening at all parts of the ventricle during the isometric period was explained by the change of shape of the ventricle to approximate a sphere which occurs early in ventricular systole. No regions were found in the normally beating heart where the myograms indicated an initial dilatation.

The intraventricular pressure of the dog shows a similar initial slow rise followed by a sudden increase of the pressure gradient. The initial period however lasts only about 0.02 sec. and the pressure rise during it is somewhat greater than in the turtle. Myograph curves recorded from certain regions of the right and left ventricles of the dog show a close resemblance to those recorded from the ventricle of the turtle. The onset of contraction is sharply defined and occurs at various times during the initial period of slow rise of intraventricular pressure. Examples of curves of this type are given in figures 1, 2 and 3. The upper curve is the myogram, shortening of the local region of muscle being indicated by a downstroke. In figures 1 and 2 the myograph was on the right ventricle and the lower curve records pressure in the right ventricle, a rise of pressure being indicated by an upward movement. In figure 1, local contraction is recorded from a region of the ventricle contiguous to the interventricular groove. The onset of contraction is coincident with the first detectable rise of intraventricular pressure; i.e., with the start of the initial period of pressure rise. The myograph in figure 2 was on the mid-anterior surface of the right ventricle in the same heart. The onset of contraction follows the onset of the initial pressure rise by 0.0112 sec. and precedes the end of this period by 0.0053 sec. Figure 3 is a myogram from a region on the left ventricle near the groove and a record of pressure in the left ventricle. The onset of contraction follows the onset of the initial rise of pressure by

0.0076 sec. and precedes the occurrence of the sharp increase in pressure gradient by 0.0124 sec. In all figures the middle line of dashes represents time intervals of 0.04 sec.

Myograms of the type described above are the rule in records obtained from near the interventricular groove, particularly on the right ventricular side. There is experimental evidence to indicate that these are the earliest



Figs. 1–10

surface regions of the two ventricles to enter into contraction and would be expected to be least influenced by local contraction of muscle occurring elsewhere. Other regions, as shown in figure 2, may however exhibit the same clear onset of local contraction.

In certain myograms, however, recorded from the surface of the ventricles of the dog, in contrast to those from the ventricle of the turtle, the

onset of local shortening is not clearly defined. The region may show a dilatation lasting throughout the isometric period of the ventricle as shown in figure 4. These myograms resemble those recorded by Tennant and Wiggers (3) in a previous study. At still other regions, the onset of shortening may be indicated, but the shortening is quickly replaced by a dilatation of the region until after the isometric period is completed (fig. 5).

The relation of the onset of shortening of various regions to the isometric period of the ventricle is shown by records in which myograms are recorded from the surface of the right ventricle along with pressure from the pulmonary artery. At regions near the interventricular groove, local shortening may reach almost its maximum before the end of the isometric period of the ventricle, as indicated by the rise of pressure in the pulmonary artery. In figure 6, the myogram is from a region on the anterior surface of the right ventricle contiguous with the groove. The onset of shortening occurs 0.0558 sec. before the start of the rise of intrapulmonic pressure. The end of the rapid period of shortening and the end of the isometric period are coincident. At other regions, particularly those along the base of the ventricle and near the conus, shortening may be in large part delayed until the end of the isometric period. In figure 7, the myogram is from a region near the conus. The onset of rapid shortening in this region does not occur until about 0.01 sec. after the end of the isometric period, and the occurrence of full shortening is coincident with the maximum pressure in the pulmonary artery.

It would hence appear clear that at certain local regions of the surface of the ventricles of the dog, shortening of the muscle occurs unimpeded by the rise of intraventricular pressure, and bears the same time relation to the intraventricular pressure as in the turtle. At other regions, however, contrary to the situation in the turtle, shortening is either more or less impeded by the rise of intraventricular pressure resulting from contraction of ventricular muscle in other regions, or the muscle itself has a slower rate of contraction.

The relation of the peak of the differential curve to the onset of shortening. Simultaneous recordings of myograms and differential potential curves from regions on the surface of the ventricles of the dog show that at certain regions at least, the same relation exists between the onset of shortening and the main peak of the differential curve as we have previously found in the turtle. In regions from which myograms can be obtained which indicate clearly the onset of shortening, the start of this process is synchronous or nearly so with the main peak of the differential curve. An example is given in figure 8. The upper curve records the potential at the differential electrode. The lower curve is the myogram from the same region. The records were made from a region on the right ventricle close to the groove. The onset of shortening is synchronous with the main

peak of the differential curve. In 30 similar records, obtained from 5 experiments, the onset of shortening and the main peak of the differential curve were synchronous in 16. In 10, the main differential peak appeared to precede and in 4 to follow the onset of shortening by intervals of less than 2 milliseconds. As an average of all the records, shortening followed the main differential peak by 0.4 millisecond, a figure which is probably not significant.[2]

As a corollary to the relation of the myograph and differential curves, it is found that the main peak of the differential curve has the same relation to the intraventricular pressure as does the onset of local shortening to this pressure. Simultaneous recordings of differential potentials and intraventricular pressure show that the main peak of the differential curve derived from various surface regions falls within the initial period of slow rise of pressure. An example is given in figure 9. The differential potential-time curve is from a region on the right ventricle a short distance above the groove. The main peak of the differential curve occurs 0.0093 sec. after the start of the initial period of slow rise of pressure in the right ventricle, and 0.0129 before the end of this period. At certain regions contiguous to the groove, the main peak of the differential curve and the start of the rise of intraventricular pressure may be simultaneous.

It is thus possible to show that at certain regions the same fundamental relation exists between the mechanical event of shortening and the electrical state, as expressed by the main peak of the differential potential-time curve on the surface of the dog's ventricles, as may be demonstrated at all regions on the ventricle of the turtle. It has not been possible to demonstrate this relation at all regions on the surface of the ventricle of the dog because at certain regions myograms fail, as has been discussed in the previous section, to reveal the instant at which the local region of muscle enters into the shortening process.

Relation of unipolar and differential potential time curves. From most regions of the surfaces of the right auricle and ventricle, the unipolar curve is diphasic, with the first peak in the positive direction. The interval between the two peaks in the dog is of the order of 0.01 sec. or less, as

[2] In order to estimate the significance of differences in time relations of curves measured under the conditions of the present work, a record was made of simultaneous recording of differential and unipolar potential curves from a heart in which 50 successive cycles were recorded. The record was made at a recording speed such that one millimeter along the time axis corresponded to 9.0 milliseconds. Measurements were made between a sharp peak on each curve in the 50 cycles and submitted to statistical analysis. The principal results, expressed in milliseconds, are as follows. Mean, 9.18 ± 0.047. Standard deviation, ±0.33, ±0.033. Coefficient of variation, 3.8 per cent. Three times the standard deviation is approximately one millisecond. This degree of variation corresponds to a difference of about 0.11 mm. on the record.

compared to an interval of about 0.04 sec. in the turtle. It is in this interval that the maximum time gradient, i.e., the most rapid rate of change of potential occurs in all but a few cases. Occasionally the most rapid time gradient occurs on the distal limb of the negative peak or on the proximal limb of the positive peak. At a few regions of the right auricle and ventricles, the potential grows to a positive or negative value and maintains this polarity throughout the QRS period. Notably is this true over the upper part of the sulcus terminalis of the right auricle. As previously reported by Wilson, Macleod and Barker (4), this region gives unipolar curves which are monophasic in the negative direction. In monophasic unipolar curves the growth of the potential is rapid, the decline slow, the maximum gradient occurring on the proximal limb.

The differential curve from the dog's heart shows consistently the same relation to the unipolar curve that we have previously reported for the tortoise heart; the main peak of the differential curve occurs coincidentally with the period of maximum gradient on the unipolar curve. Differential peaks occur during gradients of the unipolar curve, and their magnitudes are proportional to these gradients. An example of the usual type of curve is given in figure 10. Small downwardly directed peaks occur on the differential curve coincident with the small gradients associated with the development of the initial positive potential and with the decline of the final negative potential of the unipolar curve. The maximum peak on the differential curve falls approximately midway between the positive and negative peaks of the unipolar curve during the period of most rapid change of potential.

Discussion. That different regions of the right auricle of the dog's heart start shortening at different time instants has been shown by C. J. Wiggers (5) and by Lewis, Feil and Rothschild (6). The former investigator used a small mechanical myograph with optical recording and developed the conception of *fractionate contraction* of different regions as contrasted with the contraction of the organ as a whole as recorded by the usual suspension methods. Lewis, Feil and Rothschild worked with a grid of threads attached at one end to the auricular wall. Local contractions were indicated by approximation of adjacent threads as determined by photographic recording. That fractionate contraction characterizes the mechanical activity of the ventricles also, is evident from the experiments reported in the present communication.

With reference to the relation which exists between the onset of fractionate contractions, intraventricular pressure and the electrical state in the region, the following statements may be made. From experiments on turtle hearts, reported previously, and from the experiments on dog hearts, reported in the present communication, two important relations have been established for all surface regions of the ventricles of the turtle and the dog.

These are, first, that the main peak of the differential potential-time curve from all surface regions occurs during the period of initial slow rise of intraventricular pressure and second, that peaks on this curve coincide with gradients on the unipolar curve recorded from the same region and are of a magnitude proportional to the magnitude of the gradient. It has been further established, for all surface regions of the turtle's ventricle and from many but not all regions of the dog's ventricles, that the main peak of the differential curve is coincident or nearly coincident with the onset of fractionate contraction and that the onset of fractionate contractions from the various surface regions falls within the initial period of slow rise of intraventricular pressure. The inability to demonstrate these relations for all surface regions on the dog's ventricle is due, as stated above, to interference with the onset of the shortening process in certain regions. No exceptions from the relations noted have been found in any region from which satisfactory curves of shortening have been obtained.

The experimental results, we believe, clearly justify the conclusion that, in general, there is a close relation existing in cardiac muscle between the onset of the contraction process in any region and the presence of an electrical state which is defined by the differential potential-time curve recorded from this region. The maximum peak of this curve which is coincident or nearly coincident with the onset of contraction, signals the maximum flow of electric current and the maximum time rate of change of current[3] in the region from which it is derived, established by a potential gradient existing between neighboring regions at which the potentials are respectively above and below the potential of the resting muscle. This instant is also characterized by the most rapid time rate of change of potential in the region, as shown by the unipolar potential-time curve.

CONCLUSIONS

Different local regions of the surface of the ventricles of the dog's heart start shortening at different time instants. The occurrence of mechanical activity, as indicated by the onset of the fractionate contraction in any region, is coincident with or separated by a brief interval from the occurrence of maximum flow of electric current and maximum time rate of change of current, resulting from a potential gradient established between neighboring regions in which the potential is respectively above and below the potential of resting muscle. This instant is signalled by the occurrence of the main peak of the differential potential-time curve and by the occurrence of the maximum gradient on the unipolar potential-time curve derived from the region that is entering into activity.

[3] It is possible that the rapid time rate of change of current associated with the large gradients immediately preceding and following the apex of the peak, may be interrupted for a brief interval. This interval, if it exists, must be so small, however, that it has no practical significance in the present work.

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A COMPARISON OF THE HISTAMINE CONTENT OF BLOOD AND BONE MARROW[1]

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It has been shown that the white cell layer of centrifuged unclotted blood from normal animals contains most of the histamine found in blood (Code, 1936; Bram Rose, 1939). Further study has indicated that, of the cells comprising the white cell layer, those originating in the bone marrow are richest in histamine (Code, 1937a; Zon, Ceder and Crigler, 1939). In this investigation an attempt has been made to determine whether or not the cells of the blood contain the histamine when they leave the bone marrow by comparing the histamine content of the blood with that of the bone marrow. If the bone marrow were free of histamine it might be concluded that the cells obtained their histamine after leaving the marrow.

METHODS. As a routine, animals were killed instantly by a blow or a shot to the head. Blood was taken before or immediately afterward from the heart. In some instances ether or nembutal anesthesia was used. The two femora were removed, split or partly sawed and then split in two and samples of marrow taken and weighed. The entire operation was carried out without interruption. In order to detect any abnormality of the white blood cells, white cell counts were made of the blood and smears of blood and imprints of marrow were taken for examination. The smears and imprints were stained with Wright's stain. In all differential white cell counts of the blood smears at least 300 cells were counted. In most instances the lymphocytes and monocytes were not recorded separately. In the course of this investigation blood and bone marrow were taken from guinea pigs, rabbits, cats, dogs, one horse and one cow.

A procedure (Code, 1937b) modified after the original Barsoum and Gaddum (1935) method was used for the estimation of histamine in both blood and bone marrow. Duplicate samples of 5 cc. blood were taken for extraction except in one instance with the guinea pig when insufficient blood was obtained for duplicate estimations. In the guinea pig, rabbit

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and cat the entire femoral bone marrow was easily shelled out as a solid red clot which assured a uniform sample from each bone. Because of the small quantity of marrow obtained from the guinea pig the samples for the two femora were weighed and extracted together, but in the case of the rabbit and cat they were often extracted separately. With the dog and the large domestic animals, uniform samples containing red marrow were not obtained because of the spotty distribution of the islets of red tissue and the difficulty in removing the marrow from the bony spicules. Yellow marrow which to the naked eye was free of red tissue was obtained in abundance from the horse and cow.

When extracts were prepared from bone marrow certain additions to the usual procedure were necessary. In order to insure uniform extraction and complete protein precipitation, the marrow was thoroughly ground with clean sand and a considerable excess of trichloracetic acid slowly added. Droplets of fat could often be seen in the trichloracetic acid filtrates of yellow marrow. The excess fat was removed by shaking once with ether.

In some instances, as a check upon the nature of the substance estimated, final water and alcoholic extraction of both the blood and bone marrow was made for comparison. It has been found that the recovery of histamine added to blood is often low when final alcoholic extraction is used (Code, 1937b). To insure more complete recovery the procedure indicated by Anrep, Barsoum, Talaat and Wieninger (1939) of frequently repeated extractions with relatively large amounts of alcohol has been followed. The amount of alcohol used in the present study for extraction per unit volume of blood was twice or more than twice that advised by Barsoum and Gaddum.

All histamine assays were made using the lower ileum of the guinea pig suspended in Tyrode' solution containing atropine sulphate. The estimations are given in terms of gamma histamine base. The quantity of blood used for extraction was measured by volume but in order to make the determinations more comparable with those of the bone marrow the blood values have been computed to gamma per gram by using an average specific gravity value for blood of 1.050 (Barbour and Hamilton, 1924; Nice and Katz, 1935).

Results. *Guinea pig.* In the guinea pig the bone marrow contained from 27 to over 170 times as much histamine as the blood. The bone marrow histamine ranged from 10.4 to 22.2 γ per gram with an average concentration of 15.2 γ per gram (table 1). With the exception of one animal the histamine content of the blood ranged from 0.225 to 0.067 γ per cubic centimeter. These values are within the maximal and minimal concentrations of 0.280 and 0.066 γ per cubic centimeter noted in an earlier study of the blood of 20 normal guinea pigs (Code, 1939). The exception

TABLE 1

A comparison of the histamine content of the blood and bone marrow in the guinea pig, rabbit and cat

ANIMAL	NO.	WHITE BLOOD CELL COUNT: Total	Differential per cent: N	E	B	L & M	BLOOD HISTAMINE		MARROW HISTAMINE	M.H./B.H.
		thousands					*γ/cc.*	*γ/gram*	*γ/gram*	
Guinea pig	1	10.6	51.0	3.3	0.7	45.0	0.067	0.064	10.4	162
	2	8.0	30.0	9.0	0.3	60.7	0.167	0.159	22.2	140
	3	15.5	39.0	4.7	0.7	55.6	0.225	0.214	12.5	58
	4	14.3	31.7	2.7	0.0	65.6	0.069	0.066	11.8	179
	5	10.1	57.2	0.7	0.7	41.4	0.871	0.830	22.2	27
	6						0.160	0.152	15.0	99
	7						0.178	0.170	12.0	71
Average									15.2	
Rabbit	1	7.8	19.0	1.2	3.2	76.6	2.22	2.12	10.00	4.7
	2	8.8	22.7	0.3	1.7	75.3	2.00	1.91	6.66	3.5
	3	7.5	42.3	1.3	0.4	56.0	1.95	1.86	8.42	4.5
	4	7.7	39.3	0.4	2.3	58.0	2.50	2.38	11.05	4.6
Average									9.03	
Cat	1	11.3	42.0	5.0	0.0	53.0	0.044 0.044† 0.044*	0.042	3.05 3.37	76
	2	15.6	47.3	4.0	0.3	46.4	0.047 0.045	0.044	13.12 12.90*	296
	3	14.6	53.0	6.0	0.0	41.0	0.053 0.057*	0.052	4.00	77
Average									6.74	
	4	28.4	37.0	2.0	0.3	60.7	0.436 0.414*	0.405	30.77 32.00	77

Explanatory notes. The percentage of neutrophils, eosinophils, basophils and lymphocytes and monocytes found in the differential count is given under the columns N, E, B and L and M respectively. The histamine concentration in the duplicate samples of blood and marrow are shown for the cat while in the case of the other animals the duplicates were averaged to give the values tabulated. The figures in the last column were obtained by dividing the blood histamine (B.H.) into the marrow histamine (M.H.). The blood sample marked † was ground with sand in order to test the effect of this procedure on the histamine estimation. Final alcoholic extraction was used in the samples marked with an asterisk.

(animal 5, table 1) had a blood histamine of 0.871 γ per cubic centimeter. None of the observations made offered an explanation for this abnormally high value.

The rabbit. In the four rabbits tested the bone marrow contained from 3.5 to 4.6 times as much histamine as the blood. The bone marrow histamine content was between 6.66 and 11.05 γ per gram with an average concentration of 9.03 γ per gram (table 1). The white cell counts and blood histamine values were within normal values.

The cat. In four cats the histamine content of the bone marrow was consistently higher than that of the blood (table 1). The bone marrow of three of those animals was almost 76 times richer in histamine than the blood, while in one animal the ratio was 296 (cat 2, table 1). In one animal there was a leucocytosis of 28,350. The remaining three normal cats gave blood histamine values between 0.04 and 0.06 γ per cubic centimeter and bone marrow concentrations of 3 to 13 γ per gram with an average of 6.74 γ per gram. The blood of the animal showing the leucocytosis contained 0.4 γ histamine per cubic centimeter, or about 10 times as much histamine as the normal members of the group. The concentration of histamine in the bone marrow was more than twice the highest value obtained in the normal animals. This finding emphasizes the importance of a careful examination of blood when determining normal blood histamine values.

The dog. The histamine content of the bone marrow of the dog always exceeded that of the blood (table 2). The bone marrow contained 0.07 y to 0.96 γ per gram and in most instances, as is usual in the dog, the blood was free of histamine. The dog's bone marrow histamine content was considerably lower than that of the guinea pig, rabbit or cat. This difference in histamine concentration was associated with a distinct difference in the character of bone marrow in these animals. In the guinea pig, rabbit and cat the bone marrow had the appearance of homogeneous red clot which could be shelled out of the bone without great difficulty. In the dog the marrow was composed of a mixture of red, cellular material and yellow fatty substance which was closely adherent to the bony spicules and could not be removed in a block. In two instances in the dog an attempt was made to separate the red from the yellow marrow. The separation was not complete. The samples obtained were, at best, either predominantly red or yellow and for that reason were referred to as reddish or yellowish marrow respectively. The reddish marrow contained more histamine than the yellowish marrow (table 2).

Horse and cow. Because the yellowish marrow of the dog contained some histamine it seemed possible that the tissue forming the framework of the marrow contributed to the histamine content of the whole marrow. Pure yellow marrow was obtained from the femora of a horse and a cow.

One sample of yellow marrow from the horse contained a quantity of histamine which could just be detected. All other samples of yellow marrow from the horse and cow were free of histamine (table 2). In contrast to the yellow marrow, the red marrow obtained from these animals contained significant quantities of histamine. The greater proportion of red

TABLE 2

A comparison of the histamine content of the blood and bone marrow in the dog, horse and cow

ANIMAL	NO.	BLOOD HISTAMINE		MARROW HISTAMINE, γ/GRAM		
				Reddish	Yellowish	Yellow
		γ/cc.	*γ/gram*			
Dog	1	0.018	0.013	0.958	0.781	
		0.010				
	2	0.00	0.00	0.500	0.215	
		0.00			0.215*	
	3	0.00	0.00		0.080	
		0.00			0.070	
	4	0.00	0.00	0.400	0.277	
		0.00		0.384*	0.200*	
		0.00*				
		0.00*				
Horse	1	0.027	0.029	2.00		0.017
		0.033		2.06		0.00
Cow	1	0.015	0.014	0.027		0.00
		0.015				0.00
		0.015				

Explanatory notes. The histamine values for the duplicate and triplicate samples of blood and bone marrow are recorded. Activity less than 0.01 γ per cc. or gram was regarded below the limit of accurate histamine estimation and recorded as 0.00 γ. Most samples so indicated were actually free of any recognizable histamine activity. Final alcoholic extraction was used in the samples marked with an asterisk. The white blood cell counts for the dogs were normal. The blood cells were not counted in the horse and cow.

tissue found in the femur of the horse was associated with a higher concentration of histamine than that found in the reddish marrow of the cow (table 2).

Final alcoholic extraction. In the final stages of the preparation of the extracts of blood and bone marrow either water or alcohol was used to extract the dried residue obtained after acid boiling. As a routine the

extraction was made with water but in three experiments in the cat and one in the dog extracts were prepared with alcohol (tables 1 and 2). In these instances water extraction was carried out on identical samples for comparison. In order that differences in grinding the marrow with sand and trichloracetic acid might not affect the results, the marrow was treated as one batch and then the trichloracetic acid filtrate divided into equal samples, one for extraction with water, the other for treatment with alcohol.

In one instance, the histamine content of the alcoholic extract of the blood was slightly above that prepared with water although the difference was not significant (cat 3, table 1). In all other cases, the alcoholic extracts contained the same or somewhat less histamine than the water extracts. Alcoholic extractions of the yellowish marrow in one experiment gave a histamine concentration of 0.200 γ per gram while extraction with water yielded 0.277 γ histamine per gram. With this exception, agreement between the alcohol and water extracts was sufficient to allow the conclusion that the active substance estimated was the same in the two extracts.

Discussion. In all animals tested the histamine content of the bone marrow was greater than that of the blood. The whole blood histamine concentration however may not be the best basis for comparison since it includes the plasma which is poor or lacking in histamine and not present to the same extent in the marrow. The plasma of the blood of the animals used in this study is seldom more than two-thirds of the whole blood. When this ratio is used to correct the histamine content of the blood to represent gamma histamine per gram of cells the marrow still contains more histamine than the blood, although in the rabbit the difference is hardly significant.

The histamine content of the red marrow obtained from the guinea pig, rabbit and cat was greater than that of the mixed yellow and red marrow found in the dog, horse and cow. In the horse and cow the red cellular marrow contained the histamine while the inactive fatty marrow and its supporting frame work were free or practically free of histamine. The results indicate that the portion of the marrow most closely concerned with the production of blood cells contains the histamine.

Since the white cell layer of centrifuged normal blood contains most of the histamine found in blood, it seems likely that the histamine in red marrow is associated more with the white cell than with the red cell producing elements. This contention receives support from the finding that in myelogenous leukemia when immature white cells are present in the blood stream the blood histamine is elevated (Marcou, Parhon and Comsa, 1936; Code and Macdonald, 1937).

The constituents of the white cell layer of centrifuged blood arising in

the bone marrow are the granular or myeloid leucocytes and the platelets. Both types of cells may contain histamine. In most animals accumulated evidence indicates the myeloid leucocytes as the cells containing histamine in the blood. In the rabbit, however, unlike man and the horse, platelet deposits obtained by differential centrifugalization of the blood may be rich in histamine (Code, 1937a). Further, Zon, Ceder and Crigler (1939) have found a drop in the blood histamine of rabbits when the platelet material of the blood was reduced by the administration of antiplatelet serum. In the rabbit it seems possible that the property of the myeloid leucocytes to carry histamine is shared by the other constituent of the white cell layer which arises in the bone marrow, namely, the platelet.

The alcohol-insoluble, water-soluble active factor found by Anrep, Barsoum, Talaat and Wieninger (1939) in the red cells of dogs in Egypt was not encountered in this study. The good agreement between the histamine concentration of alcoholic and water extracts of similar samples of blood and marrow indicated only histamine was being estimated. It seems unlikely that the alcohol-insoluble, water-soluble factor found in Egypt has even played a significant rôle in the histamine studies carried out with the modified method in England and the United States. During the past four years red cells of dogs in England and the United States have been frequently tested using a final water extraction and in nearly all instances the extracts have been found to be free of histamine, indeed free of a significant quantity of any substance which would contract the guinea pig ileum (Code, 1937a, 1939).

The satisfactory yield of histamine when final alcoholic extraction was used in this study was believed due to prolonged extraction with excess alcohol. Using alcoholic extraction as directed in the original Barsoum and Gaddum method (1935), Code (1937b) obtained unsatisfactory agreement between duplicate extracts of 10 cc. of blood in about one-third of the samples and noticed a failure of the alcoholic extraction of this method to yield a consistently satisfactory recovery of histamine added to blood. The difficulty of obtaining a sufficient recovery with alcohol seems partly mechanical. In the isolation of histamine from white blood cells Code and Ing (1937) used alcohol in eight instances to extract different batches of the dried residue obtained from trichloracetic acid filtrates. The residue was generally a solid cake on the side of the flasks and this had to be broken and subjected to repeated, prolonged extraction to give complete recovery of histamine. In the routine preparation of extracts of blood the residue obtained after drying the boiled trichloracetic acid filtrates often forms a thin hard layer on the flask the bulk of which is insoluble in alcohol. Anrep, Barsoum, Talaat and Wieninger (1939) have repeated the work of Code in testing the recovery of histamine added to blood when alcoholic extraction is used. They have not stated whether they used 10 cc. blood as required

by the Barsoum and Gaddum method or 5 cc. as suggested in the modified procedure. Utilization of the smaller volume of blood, by reducing the quantity of residue, facilitates the extraction. With the use of larger quantities of alcohol and more frequent extraction than originally advised by Barsoum and Gaddum, Anrep et al. have obtained the complete recovery of histamine added to blood. In the present study when prolonged repeated extraction with relatively large volumes of alcohol was used a satisfactory recovery of histamine was also obtained.

SUMMARY

In guinea pigs, rabbits, cats, dogs and one horse and one cow the histamine content of the femoral bone marrow exceeded that of the blood. The histamine in marrow was found associated with the red or cellular portion of the tissue. Pure yellow marrow was free or almost free of histamine. It seems possible that the cells of the blood containing histamine may carry it with them as they leave the marrow. The use of alcohol in the extraction of blood for histamine estimation is discussed.

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ON THE REGULATION OR HOMEOSTASIS OF THE CHOLIC ACID OUTPUT IN BILIARY-DUODENAL FISTULA DOGS[1,2]

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The occurrence of an entero-hepatic circulation of bile salts, first demonstrated by Schiff (1), is established. However, relatively little is known concerning the method by which the cholic acid output is regulated. It has been postulated that some regulatory mechanism must exist in the animal organism which prevents the accumulation of bile salts (2, 3).

In previous studies with bile fistula dogs we have made two observations which appeared of special significance for the regulation or homeostasis of the bile salt output. First, we have noted in 250 experiments on 75 biliary fistula dogs that, if no bile salts are given, the bile salt output on our standard diet attains a remarkably constant level of about 0.46 gram per 8 hour period (3, 4). Second, we observed that, when whole dog bile or bile salts were administered, a fairly constant proportion of approximately 90 per cent could be recovered as "extra" bile salts in the bile (3, 4). If it is true that a constant quantity is synthesized under constant dietary and digestive conditions, and that a constant proportion of the bile salt intake is recovered in the bile during one entero-hepatic circuit, it follows that at some time the amount lost will equal the amount synthesized, and that the bile salt output will be regulated or maintained at a constant level. That is, given an animal fed at 8 hour intervals without the return of bile, if the initial supply of bile salts is low, synthesis will gradually restore a homeostatic level, and if the initial supply is in excess, 10 per cent loss during each entero-hepatic circuit will reduce it to a homeostatic level.

In order to test the above reasoning, we have followed the course of cholic acid output during successive 8 hour periods in biliary-duodenal fistula dogs under the following three conditions: *a*, when the initial dose was only 0.46 gram (the basal output per 8 hr. period, when no bile is returned); *b*, when the initial dose was 2.7 grams, and *c*, when the initial dose was 5.7 grams.

METHODS. Seven biliary-duodenal fistula "suction" dogs were used.

[1] This work has been assisted by the E. L. Dawes Fund.

[2] This work was aided by the E. L. Dawes and the Marjorie Newman Grants.

The animals weighed between 10 and 12 kilos. The biliary fistula was similar to that employed by Rous and McMaster (5), except that the rubber catheter was placed through the cystic duct into the common hepatic duct. A small rubber tube, $\frac{1}{32}$ x $\frac{1}{32}$ inch, was placed into the duodenum, so that it extended caudalward about 6 to 8 inches. Through this tube the bile was returned into the intestine at the rate of 1 cc. per minute. The return bile was allowed to drip into the intestine usually over a period of 3 to 4 hours. To insure the most quantitative results, a small amount of suction was applied continuously to the tubing draining the biliary passages (6, 3). The amount of suction was never greater than 16 inches of water pressure. The animals were kept on suction for 24 hours a day, except for a few minutes at meal time and when they were dressed. They were fed three times a day, and each time they received one-third of the diet of "Pard" and milk (12 per cent protein, 9 per cent carbohydrate, 6 per cent fat, and a supplement of cod liver oil and dried yeast), so that their daily control output of cholic acid would approximate the amount obtained in previous studies.

The *experimental procedure* consisted of the following steps: 1. Several weeks after the operation the animal was standardized to the diet and the return of bile to insure a healthy state. 2. *Basal period.* Then, the animal was fed the diet every 8 hours but no bile was returned for from 3 to 5 days. This gave the basal output of cholic acid on the diet without the return of bile. 3. *The experimental period.* Then, the animal was given the initial dose of cholic acid in the form of dog's bile. At each meal time, or at each 8 hour interval, the bile secreted during the previous period was collected, measured, and 1 to 2 cc. removed for cholic acid analysis. The remaining volume of bile was then returned to the intestine of the animal during the first 3 to 4 hours of the next 8 hour period. Then, at the end of the next 8 hour interval, the animal was fed again, the bile measured, and after removing 1 to 2 cc. for cholic acid analysis the remainder was returned to the dog. This procedure was performed three times a day at 8 hour intervals throughout the length of an experiment. Thus, the bile salts made three entero-hepatic circulations each day.

Kocour and Ivy (6) found that, when food and bile was given every 6 hours, the volume output of bile was very constant. Schmidt et al. (3) noticed no accumulation of cholates when bile and food were fed every 8 hours. Because of these observations we chose to subject the bile salts to three circuits each day.

Calculation of the per cent recovery of cholates. During each period we know the intake and output of cholates. For example, the intake is 3.8 grams and the output is 3.8 grams for an 8 hour period. The output minus the synthesis for a 8 hour period, or $3.8 - 0.46 = 3.34$, gives the output actually obtained from the intake of 3.8 grams; 3.34 divided by 3.8 =

88 per cent recovery of the cholates introduced. Until the introduced cholates are labelled in some way, it must be assumed that synthesis remains approximately constant for each 8 hour period during the absorption of cholates and that the 12 per cent "loss" applies only to the cholates introduced into the intestine. This assumption is implicit in all of our calculations, but does not alter the observed facts or the conclusions.

RESULTS. The averaged data from the three experiments on the different initial doses of cholic acid are shown graphically in figures 1, 2 and 3. It will be seen that in each case the cholic acid output leveled off at

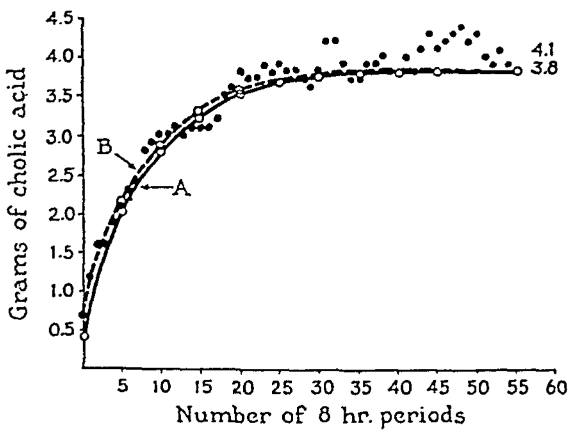

Fig. 1

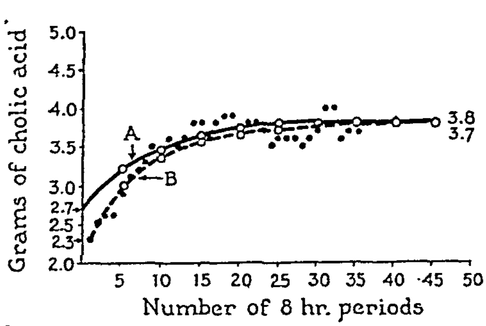

Fig. 2

Fig. 1. Left. This is the average curve of recovery (5 dogs) when the initial return of cholic acid was that amount excreted during the preceding 8 hour period, during which no bile was returned. The dots indicate the experimental recovery of each period. Curve A, the broad-line, represents the curve calculated from $O_n = B^nI + A\left(\frac{B^n - 1}{B - 1}\right)$, where $B = 0.88$; $I = 0.46$, and $A = 0.46$. Curve B, the broken-line, represents the curve calculated when I is the actual input of the second 8 hour period; that is, the actual initial dose is ignored and the dose given at the second administration is used.

Fig. 2. Right. This is the average curve of recovery (5 dogs) when the initial input of cholic acid was 2.7 grams. Curve A: $B = 0.88$, $I = 2.7$, and $A = 0.46$. See legend of figure 1 for formula and meaning of curve B.

approximately the same point regardless of the initial dose of bile salts. In fifteen tests the cholic acid output leveled off at 4.1 grams per 8 hour period, while in five tests a relatively constant output was reached at 3.7 grams per 8 hour period. When a large dose of cholic acid, such as 5.7 grams, was the initial dose, the curve of the cholic acid output fell to the "*homeostatic level.*" Whereas in the other initial doses, 0.46 gram and 2.7 grams, the curve rose to the "homeostatic level." It can also be seen that, when large doses of bile salts, such as 2.7 and 5.7 grams, were introduced into the intestine at a time when the animal is basal for the diet but no return of bile, the liver or intestine was unable to utilize efficiently the

intake during the first 8 hour period. However, the liver or intestine readjusted itself rapidly, and the cholic acid output either rose or fell gradually to the homeostatic level.

The data have also been analyzed mathematically. In figure 4, the output of cholic acid has been plotted against the input for each individual period of the three experiments. Obviously the points tend to form a straight line, and the data were fitted to the following straight lines, $y = bx + a$ and $x = by + a$. Both regression equations were obtained and the average of the two is plotted on the graph. The correlation

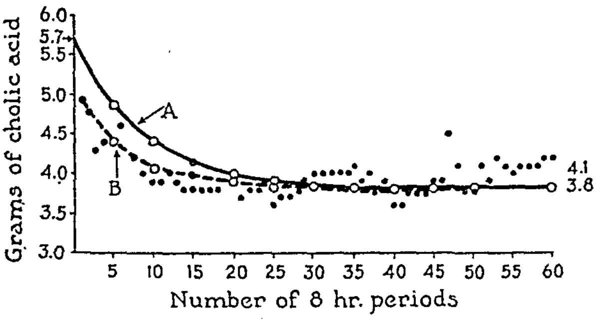

Fig. 3

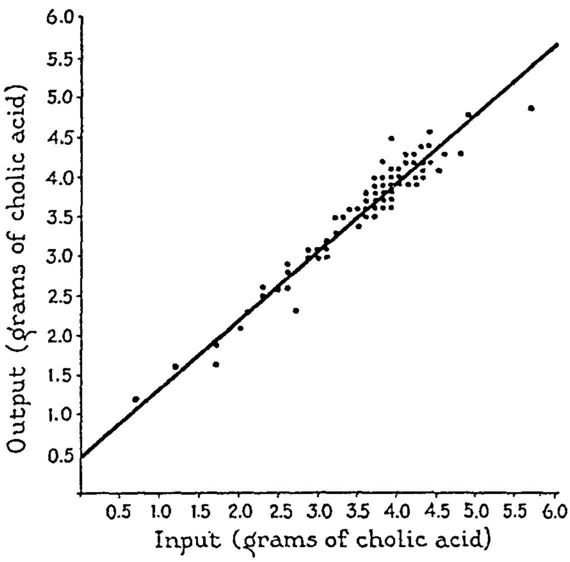

Fig. 4

Fig. 3. Left. This is the average curve of recovery (5 dogs) when the initial return of bile salts was 5.7 grams. Curve A: $B = 0.88$, $I = 5.7$, and $A = 0.46$. See legend of figure 1 for formula and meaning of curve B.

Fig. 4. Right. This shows the relation between output and input of cholates. The dots represent the experimental data. $y = bx + a$; $b = 0.8505$; $a = 0.568$; $y = x = 3.799$. $x = yb + a$; $b = 0.9015$; $a = 0.389$; $y = x = 3.956$. The straight line is the average of the two equations. Average basal output on diet alone per 8 hour period, 0.479 gram; recovery, 87.6 per cent; and homeostatic level, 3.878 grams. Correlation coefficient, 0.94.

coefficient is 0.94, which indicates a high degree of correlation between the input and the output. The final equation which shows the linear relationship between input and output has the following form: Output = input $\times$ $b + a$ where b is the slope = 0.876, and a is the point of intersection on the ordinate = 0.479.

There are several points of significance revealed by this equation. First, the high correlation coefficient (0.94) indicates the relative accuracy of the linear relationship between input and output of cholic acid, within the range studied. It should be mentioned that, if large enough doses are

given diarrhea results and will alter the relationship (3); this level was avoided in the present study. Second, the a constant, which is the output intercept, indicates that if no bile salts are administered, 0.479 gram of cholic acid per 8 hour period will be secreted in the bile. From 250 experiments on at least 75 biliary fistula dogs, we have experimentally established the control output on our diet to be 0.46 gram per 8 hour period. Thus, we have a good check of the basal synthesis on our diet. Third, the b constant, the slope, reveals that the recovery of administered bile salts is constant in these dogs at about 88 per cent. In order to calculate recovery during the return of bile, one must assume the existence of a synthesis of about 0.48 gram per 8 hours. Calculation of the individual recoveries for each period in the three experiments has given an average recovery of 88 per cent and has revealed no tendency to vary with the dosage. Thus, another good check was obtained. Fourth, the equation reveals that, if small doses are given, the output will exceed the input, and that if large doses are given the output will fall short of the input, but never by more than 12 per cent (unless diarrhea occurs). At some point between these two extremes the input will just equal the output, and solving the equation reveals this level to be 3.8 grams per 8 hours. The average output of cholic acid during the terminal 10 periods in the three experiments, when the animals had apparently leveled out, is 3.9 grams per 8 hours. Fifth, from this it follows that regardless of the initial dose, if the secreted bile is returned, a final level of approximately 3.8 grams should be reached.

The relationship between the initial dose and the course by which 3.8 grams per 8 hours is finally reached can be expressed by the following equation: $O_n = B^nI + A\left(\frac{B^n - 1}{B - 1}\right)$ in which n represents the successive 8 hour periods, O, the output of cholic acid at any period n, B, the percentage recovery of cholic acid administered during any period n, namely, 88 per cent, I, the initial dose, and A, the basal cholic acid output on the diet, or 0.46 gram per 8 hour period. The curves predicted by this equation have been drawn in figures 1, 2 and 3. It will be seen that there is fair agreement between the theoretical curves and the actual data. The broad-line curve, A, is the curve obtained when the level of the initial period of return of bile is used. The broken-line curve, B, is the curve obtained when the second period of return of bile is used. Curve B fits the experimental data best, and is to be preferred because after a 3 to 5 day period of no absorption of bile salts the liver or intestine apparently does not handle the bile salts as it does later; with the higher doses more bile salts are "lost" during the initial period. When the initial dose is low, or is the control output, the two curves are practically identical.

Discussion. The data show definitely that regardless of the initial dose of cholates, the cholic acid output progressively returns to a homeo-

static level which is determined by the basal synthesis of cholic acid from the diet used. When the initial dose of cholic acid introduced into the intestine is less than the dietary homeostatic output per 8 hour period, the cholic acid being circulated accumulates until it reaches the homeostatic level of output. This is because the rate of synthesis exceeds the rate of "loss" per 8 hour period. When the initial dose of cholic acid is greater than the dietary homeostatic output per 8 hour period, the circulating cholic acid diminishes until it reaches the homeostatic level of output. This is because the rate of "loss" per 8 hour period is greater than the synthesis. In either case the "loss" of cholate from period to period is 12 per cent, which will not be exceeded until the "efficiency level" of the liver or intestine appears to be exceeded. This occurs during the first and sometimes the second 8 hour period when bile is returned after a period of privation of bile, and also when large amounts of bile or cholic acid are given which cause catharsis or the "sluice" mechanism of the intestine to operate. When the dietary homeostatic level is reached synthesis apparently balances "loss."

Where the "loss" occurs or what process is involved has not been determined. In fact, when the animal reaches the homeostatic level of output we only know positively that output equals intake. What happens to synthesis and "loss" is unknown. Sythesis and "loss" may be equal, or synthesis may decrease and loss increase. From our observations and those of Smith, Groth and Whipple (7) the quantity and quality of the protein in the diet appear to be the most important factor determining the homeostatic level of cholic acid output. It would appear as though the homeostatic level could be maintained at 2, 3, 4, 5 and possibly 6 grams of cholic acid per 8 hour period, depending on the quantity and quality of the protein fed.

It should be noted that the equation, O_n, employed as it was in the case of curve B in the first three figures, permits the prediction of the cholic acid output for any subsequent period, when the basal output per period of time on the diet is known, the average loss of cholic acid during one entero-hepatic circuit is known, and the animal is healthy and consumes with appetite the diet fed under the conditions of our experiments. The equation also makes certain implications regarding how the homeostatic level of cholic acid output is maintained in the presence of the gall bladder and also following cholecystectomy. These implications, however, must be tested by actual experiments which simulate as closely as possible the cholecystate and acholecystate condition.

SUMMARY AND CONCLUSIONS

Fifteen experiments were performed on seven biliary-suction-duodenal fistula dogs. After adjusting the dogs to an eight-hour feeding schedule without the return of bile to the intestine in order to determine the basal

output of cholic acid on the diet, three different initial doses of cholic acid in the form of cholates in dog bile were introduced into the duodenum, and the output of cholic acid determined for each successive eight-hour period. The bile formed during each preceding eight-hour period, after the initial introduction of bile, was returned to the intestine. This was repeated every eight hours for two or three weeks, the cholic acid output for each eight-hour period being determined from a 2 cc. sample.

When fed the diet we used, the dogs synthesized from 0.46 to 0.48 gram of cholic acid every eight hours, when no bile was returned to the intestine. When a certain quantity of cholic acid in the form of cholates was introduced into the intestine, 88 per cent on the average was recovered in the bile. This recovery was quite constant for various amounts, except when amounts sufficient to cause catharsis were used. When a relatively large amount of bile was introduced into the duodenum after a period of privation of bile, the mechanisms which are exposed to the bile salts were upset during the first and sometimes the second eight-hour period.

The data show that regardless of the initial dose of cholates, the cholic acid output progressively returns under the conditions of our experiment to a homeostatic level of from 3.8 to 4.1 grams per each eight-hour period. This level is chiefly determined by the basal dietary synthesis of cholic acid, since the average percentage "loss" (12 per cent) during each enterohepatic circuit apparently remains constant. When the homeostatic level is reached after about two weeks, synthesis apparently balances "loss" of cholic acid.

An equation is presented which permits the prediction of the cholic acid output for any subsequent period when the basal output per unit of time on the diet is known and the average loss of cholic acid during one enterohepatic circuit is known and the diet and frequency of feeding are kept constant.

We gratefully acknowledge the assistance of Doctors John Gray and F. T. Jung in formulating the equation.

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GLYCOGEN LEVELS IN THE ISOLATED LIVER PERFUSED WITH CORTICO-ADRENAL EXTRACT, INSULIN AND OTHER PREPARATIONS[1]

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Although many years have elapsed since the effects of endocrine extracts were first tested in the laboratory, the influence of such preparations on carbohydrate metabolism is still not at all clear. The confusion found in textbooks and monographs, the great number of original articles and symposial discussions on the subject, all offer testimony to this fact. Some conclusions regarding relationships between the adrenal cortex and carbohydrate metabolism which have been drawn by this school in the past decade have not passed without vigorous contest. With the hope that a simplified, direct attack on the problem might lend clarity to the situation, the effects of various hormones on carbohydrate levels in the isolated liver have now been investigated. Cortico-adrenal extract, desoxycorticosterone and insulin have been tested in particular, together with potassium solutions in the extract series, for their possible influence on the glycogen content of hepatic tissues perfused *in vitro* under rigidly controlled conditions.

METHODS. The animals from which the tissues were derived were lightly anesthetized with ether; the hepatic portal vein was cannulated as rapidly as possible—usually within five minutes—and the entire liver then removed to a constant-temperature bath at 37° containing isotonic Ringer's solution. The liver was perfused through the cannulated vein in different experiments with various solutions which were continuously oxygenated and also maintained at 37°. The portal vein was used as the perfusion route since it appears to supply about 75 per cent of the blood entering the liver; also, it was felt that any possible advantage that might be gained by perfusion through both artery and vein would be vitiated by increased technical complications (in small animals such as the rat and cat) and delay in starting perfusion. The success of the technique was judged mainly by the completeness with which the blood was expelled from the

[1] Grateful acknowledgment is made of aid received from the Committee on Research in Endocrinology of the National Research Council.

liver, as indicated by the quickly fading color of that organ at the outset of perfusion. When any doubt arose regarding the adequacy of the perfusion, the experimental material was discarded.

The perfusate entered the vein at an average pressure of 10 mm. Hg, with occasional brief fluctuations between 8 and 12 mm.; commonly from 10 to 15 cc. per minute were introduced. Bearing in mind that large organs tend to deteriorate rapidly when isolated from the normal circulation, and that under such conditions glycogenolysis is greatly accelerated, the period of perfusion was arbitrarily limited to 15 minutes.

Initial tissue samples were taken as soon as the liver was placed in the bath; the sampling was then repeated at 5-minute intervals. Glycogen concentrations were determined in all cases by a modified Pflüger method (Britton and Silvette, 1932). Animals in various stages of nutrition were purposely included in the study, with the thought that responses to the various perfusates might conceivably vary in livers of high initial glycogenic

TABLE 1

The effect of perfusion of the rat liver for 15 minutes with various solutions

NO. OF RAT LIVERS PERFUSED	PERFUSATE	DECREASE IN GLYCOGEN
		per cent
5	Ringer's solution	75
5	Glucose (5%) in Ringer's solution	35
5	Cortico-adrenal extract (5%) in glucose-Ringer's solution	21

content from those given by organs depleted of carbohydrate material (see tables).

RESULTS. Cat livers were used as experimental material after considerable trial with those from the rat, since glycogenesis could not be definitely demonstrated, within the time limits employed, in the latter animal. Nevertheless, the rat results were of interest. Thus, following perfusion with Ringer's solution, the rat liver showed an average decrease in glycogen content of 75 per cent (table 1) and the addition of glucose to the perfusate reduced this glycogen loss to 35 per cent. Perfusion of rat livers with Ringer-glucose solution to which cortico-adrenal extract was added reduced the loss in glycogen, however, to an average of only 21 per cent. There was thus a decrease of 40 per cent in the glycogenolytic rate when extract was used, in comparison with that of the Ringer-glucose mixture alone.

Although the above experiments gave indirect evidence of glycogenic activity in the presence of cortico-adrenal extract, observations of actual glycogenesis were of course desirable before conclusions could be drawn

TABLE 2

Glycogen concentrations in cat livers following perfusion with various solutions

CAT NO.	LIVER GLYCOGEN (GRAMS PER CENT)				
	Normal	5 minutes	10 minutes	15 minutes	Cc. perfused
A. Control groups					
1. Glucose in Ringer's solution*					
1	0.15	0.15	0.14	0.14	700
2	0.28	0.20	0.17	0.11	600
3	0.91	1.09	0.85	0.71	700
4	2.52	2.46	2.45	1.86	900
Average	0.96	0.97	0.90	0.70	725
2. Glucose in Ringer-gum solution					
5	0.27	0.36	0.29	0.26	175
6	0.45	0.40	0.44	0.46	210
7	0.53	0.57	0.42	0.40	155
8	0.95	1.02	0.77	0.78	220
9	1.14	1.19	0.87	0.65	175
10	1.42	1.99	0.92	0.85	200
11	2.66	2.78	2.36	2.19	200
Average	1.06	1.19	0.87	0.79	191
B. Experimental groups					
1. Cortico-adrenal extract in glucose-Ringer-gum solution					
12	0.20	0.32	0.34	0.29	150
13	0.24	0.26	0.70	0.49	155
14	0.31	0.38	0.53	0.66	160
15	0.63	0.77	1.14	0.94	150
16	0.75	0.90	0.79	0.82	190
17	1.55	1.95	2.60	2.59	185
18	1.58	1.84	1.93	2.56	210
Average	0.75	0.92	1.15	1.19	171
2. Cortico-adrenal extract in potassium acetate-glucose-Ringer-gum solution					
19	0.18	0.15	0.16	0.14	200
20	0.33	0.49	0.36	0.34	220
21	0.79	0.70	0.60	0.52	300
22	0.90	0.74	0.43	0.39	190
23	1.47	1.05	0.60	0.55	210
Average	0.73	0.63	0.43	0.39	224

TABLE 2—*Concluded*

CAT NO.	LIVER GLYCOGEN (GRAMS PER CENT)				
	Normal	5 minutes	10 minutes	15 minutes	Cc. perfused
B. Experimental groups—Continued 3. Desoxycorticosterone† in glucose-Ringer-gum solution					
24	0.12	0.20	0.16	0.21	175
25	0.72	0.38	0.45	0.39	200
26	1.31	0.88	0.73	0.45	210
27	2.53	2.31	2.46	2.39	180
28	3.19	2.72	2.66	2.43	200
Average	1.57	1.30	1.29	1.17	193
4. Insulin in glucose-Ringer-gum solution (a) 1 unit per 100 cc.					
29	2.51	2.44	1.73	1.95	190
30	2.90	2.84	2.40	2.42	200
(b) 2 units per 100 cc.					
31	0.29	0.21	0.22	0.34	210
32	0.33	0.29	0.24	0.22	180
(c) 10 units per 100 cc.					
33	0.73	0.37	0.35	0.39	130
34	0.91	0.75	0.25	0.28	185
35	1.10	0.94	0.93	0.89	155
Average	1.25	1.12	0.87	0.93	179

* Composition of perfusates used:

A-1. Glucose (5 per cent) in Ringer's solution.

A-2. Glucose (5 per cent) in a 7 per cent solution of gum arabic in Ringer's solution.

B-1. Cortico-adrenal extract (5 per cent) in a Ringer solution containing 5 per cent glucose and 7 per cent gum arabic.

B-2. Cortico-adrenal extract (5 per cent) in Ringer's solution in which the potassium chloride was replaced by potassium acetate in 1 per cent concentration.

B-3. Desoxycorticosterone in oil, emulsified in a Ringer solution containing 5 per cent glucose and 7 per cent gum arabic.

B-4. Insulin in the amount indicated in the table, in Ringer's solution containing 5 per cent glucose and 7 per cent gum arabic.

† Desoxycorticosterone acetate ("Cortate") in sesame oil, furnished through the generosity of the Schering Corporation.

regarding specific action of the hormone on the liver. Further experiments were therefore carried out, employing tissues from the cat. The results are summarized in table 2.

It is apparent that no significant glycogenesis was demonstrable in any

of the experiments other than those in which cortico-adrenal extract was utilized in the perfusing fluid. A slight rise in glycogen was observed during the first five minutes of perfusion with the glucose-gum-Ringer solution. This action was transitory, however, the average glycogenic level dropping to 75 per cent of the initial value within 15 minutes (table 1, A2).

Perfusion with a similar solution to which cortico-adrenal extract was added in 5 per cent concentration yielded results indicative of glycogenesis in all instances. Following only five minutes of perfusion an average rise in glycogen content of 22 per cent was observed; the glycogen building rate increased more rapidly thereafter, and at the end of 10 minutes a 53 per cent average increase had taken place. Synthesis of glycogen for the 15 minutes' perfusion period averaged the highest of the whole group (table 2, B1).

Potassium acetate solution given with cortico-adrenal extract produced a strikingly different result: glycogenesis was prevented, and indeed losses of carbohydrate from the liver were observed (table 2, B2). In the average percentage changes in glycogen content from the initial levels shown in figure 1, it is interesting to note that the curves obtained by perfusion with cortico-adrenal extract and extract plus potassium salt are approximately mirror images.

Perfusion of the liver with desoxycorticosterone was surprisingly ineffective in promoting glycogen formation. The average figures for this group showed instead considerable glycogen loss (table 2, B3).

Insulin failed to bring about carbohydrate storage in the liver in all cases; on the contrary, there was an average fall in glycogen content of about 30 per cent during these experiments. Three different concentrations of the hormone were tested (table 2, B4). It is of course possible that longer perfusion periods of different concentrations of insulin might modify these results.

Discussion. The remarkable ability of extracts of the adrenal cortex to build up glycogen in the excised liver, shown in the present paper and an earlier preliminary report (Corey and Britton, 1940), would appear to clinch the arguments of many years past and force the conclusion that the adrenal cortex elaborates a specific glycogenetic hormone. To this belief most of the results of this laboratory have indeed led for more than a decade. It is now observed that this glycogen-forming or glycopexic hormone of the adrenal cortex is able to function as an independent, specific agent, on the isolated organ. It is able to act directly and rapidly and may raise the carbohydrate content of the liver 50 or even 100 per cent in 10 or 15 minutes. Further, it is effective in the presence of low or even high initial liver glycogen concentrations; in one case the value rose from 0.24 to 0.70 gram, and in another from 1.55 to 2.60 grams per cent,

each within a 10-minute experimental period. A report by Seckel (1940) indicates that glycogenolysis in the excised liver may be inhibited by extracts of the adrenal cortex.

Addition of potassium acetate to the perfusing fluid apparently rendered cortico-adrenal extract, present in the same solution, inactive as far as glycogenetic activity is concerned. When the percentage changes in glycogen concentration are considered (fig. 1), it is apparent indeed that potassium perfusion resulted in the lowest hepatic glycogen levels which

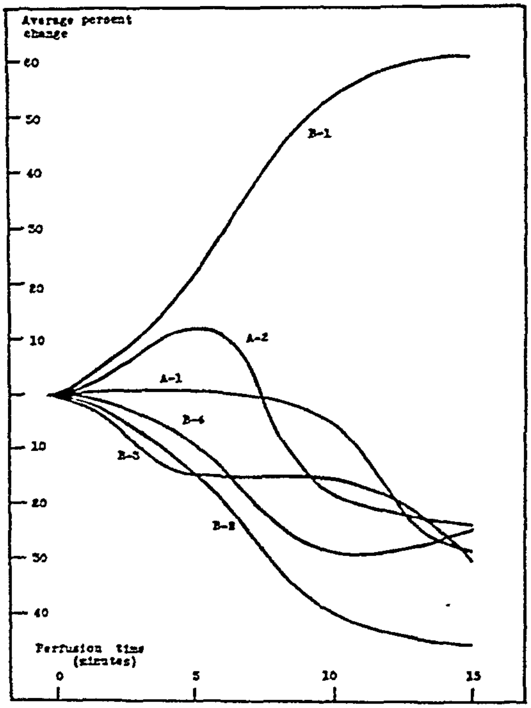

Fig. 1. Showing the average percentage change in the glycogen content of perfused cat livers. The legends correspond to the various divisions of table 1, thus: *A-1*, glucose-Ringer's solution; *A-2*, glucose-gum-Ringer; *B-1*, cortico-adrenal extract in glucose-Ringer-gum; *B-2*, cortico-adrenal extract in potassium-acetate-glucose-Ringer-gum; *B-3*, desoxycorticosterone in glucose-Ringer-gum; *B-4*, insulin in glucose-Ringer-gum solution.

were encountered. A definite antagonism is thus indicated between the action of the potassium salt and the cortico-adrenal hormone. Membrane or osmotic effects may of course be involved, as discussed in an earlier paper (Britton, Silvette and Kline, 1938).

Inability to demonstrate hepatic glycogenesis in any of the control or other experiments is in contrast very striking. Tests with different concentrations of insulin (1, 2 and 10 units per 100 cc. perfusing fluid) which were carried out appear to be definitely negative. Under similar conditions cortico-adrenal extract was highly effective.

Desoxycorticosterone also produced no positive influence on the liver glycogen levels *in vitro*, which is in keeping with correlated observations in this and other laboratories. This substance obviously lacks, therefore, an important (glycogenetic) constituent found in whole extracts of the adrenal cortex. It is of special note, on the other hand, that when the isolated liver is perfused with whole cortico-adrenal extract it is able to build up promptly large amounts of glycogen. This insulin fails to do. Considered in relation to somewhat similar results on the intact animal given in the following paper (Britton and Corey, 1941), these findings appear to be highly significant.

SUMMARY

Cortico-adrenal extract perfused through the rat liver with glucose proved more effective in the prevention of glycogenolysis than did glucose alone.

In the perfused cat liver *in vitro* large increases in glycogen content were brought about by the use of cortico-adrenal extract with Ringer-gum-glucose solution. Elevations of 50 to 100 per cent above the pre-perfusion glycogen levels occurred in some instances within 10 or 15 minutes. No other perfusates tested had this effect; in contrast, there usually occurred marked diminution in the glycogen values.

Addition of potassium acetate to perfusion fluids containing cortico-adrenal extract prevented the glycogenetic action of the extract.

Desoxycorticosterone acetate did not stimulate glycogenesis in the isolated cat liver.

Glycogenesis could not be demonstrated in cat livers perfused with glucose solutions containing insulin in the several different concentrations employed in these experiments. Instead there occurred an average glycogen decrease of about 30 per cent under insulin dosage, while an average rise of 60 per cent was effected by extracts of the adrenal cortex.

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PANCREATIC AND CORTICO-ADRENAL INVOLVEMENT IN CARBOHYDRATE REGULATION[1]

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Toward the end of an extended review written ten years ago it was stated that the adrenal cortex was apparently "concerned with the storage and utilization of carbohydrates, and possibly with some phase of protein metabolism" (Britton, 1930). Shortly after this it was shown that extracts of the adrenal cortex produced hyperglycemia in normal and adrenalectomized animals, and that the blood-sugar-raising ability was a direct function of the amount of hormone injected and of the elapsed time (Britton and Silvette, 1931a). The effects of cortical preparations were produced by oral as well as other modes of administration (Britton and Silvette, 1931b). Evidence of remarkable increases in liver and muscle glycogen, effected by cortico-adrenal extract in normal and adrenalectomized animals, was also brought forward. The terminal stages of adrenal insufficiency were characterized in most animal types by severe hypoglycemic convulsions, it was observed, and restoration from extreme prostration by adrenal extract was found to synchronize with increases in blood glucose (Britton and Silvette, 1932). In face of much opposition to this earlier work the Virginia school steadily extended and corroborated these results, and within the past few years rather general support for their observations has been advanced.

METHODS. The relative activities of the pancreas and adrenal cortex in carbohydrate regulation have been particularly investigated in the present studies. Carbohydrate levels in adrenalectomized, pancreatectomized, and adreno-pancreatectomized cats have been determined under different experimental conditions. Adrenalectomy and pancreatectomy were performed in single-stage operations; adreno-pancreatectomy was carried out in two stages in earlier work, but equally good results were achieved in later experiments with a single quick operation, lasting about 15 minutes.

The influence of glucose feeding and injection, in some cases accom-

[1] Grateful acknowledgment is made of aid received from the Committee on Research in Endocrinology of the National Research Council.

panied by insulin or cortico-adrenal extract, has been noted and compared with conditions in operated and normal untreated animals. Some early tests were made of the effects of the hormone preparations alone, but it became apparent that the utilization of glucose served to emphasize greatly the glycogenetic possibilities. In different series the effects of the hormones over periods of a few days as well as over several hours have been observed. Glucose solutions were made up in normal saline.

Analyses of blood glucose were made by the Folin-Malmros method (1929), and glycogen by a modified Pflüger procedure (Silvette and Britton, 1932). Three samples were taken from the heart for analysis—one

TABLE 1

Carbohydrate levels in adrenalectomized cats given glucose orally over various periods

(Glucose, 5 per cent solution in 0.9 per cent saline, 7 per cent body weight, twice daily)

CAT NO.	PERIOD OF TREATMENT	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	HEART GLYCOGEN		
					Apex	Body	Base
	days	*mgm. per cent*	*per cent*	*per cent*	*per cent*	*per cent*	*per cent*
1	2	84	0.26	0.15	0.20	0.15	0.22
2	2	59	0.12	0.44	0.36	0.37	0.34
3	2	80	0.43	0.25	0.65	0.45	0.67
4	3	115	0.22	0.21	0.19	0.15	0.14
5	3	60	0.11	0.13	0.29	0.26	0.21
6	5	64	0.15	0.25	0.33	0.35	0.32
7	5	91	0.29	0.48	0.39	0.36	0.32
8	5	62	0.51	0.47	0.37	0.54	0.34
9	6	98	0.35	0.61	0.38	0.48	0.38
10	6	77	0.17	0.35	0.44	0.46	0.50
*Controls,** 10 cases, 2–6 days, averages		88	3.31	0.52	0.86	0.86	0.76

* Unoperated animals treated similarly with glucose.

each from the apex, body and base of the organ—but the small differences which were noted in the values led later to one sample only being used, from the thick mid-wall of the left ventricle.

RESULTS. *Adrenalectomized cats.* The effects on carbohydrate levels of oral administration of glucose in saline solution over different periods of days are shown in table 1. Unoperated control cats thus treated with glucose stored up large quantities of glycogen in the liver, and skeletal and cardiac muscle. Adrenalectomized animals treated similarly showed very low carbohydrate levels in all tissues; even after the long-continued (6-day) periods of glucose administration, no significant glycogen storage occurred. Similar negative results have been noted repeatedly after intraperitoneal

injection of glucose solution over shorter periods. During the experiments, it should be observed, the operated animals remained in apparently good condition.

Cardiac glycogen levels for different parts of the heart are given. All indicate the severe disability of the adrenalless animal to synthesize

TABLE 2

Effects of insulin given with glucose on carbohydrate levels under different conditions

CONDITIONS	CAT NO.	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	HEART GLYCOGEN
		mgm. per cent	*per cent*	*per cent*	*per cent*
A. *Adrenalectomized cats*					
200 mgm. glucose + 2 units per kilo insulin per hour	11	62	0.13	0.37	0.32
	12	50	0.41	0.50	0.60
	13	51	0.12	0.43	0.65
300 mgm. glucose + 2 units per kilo insulin per hour	14	54	0.18	0.61	0.79
	15	51	0.39	0.36	0.57
300 mgm. glucose + 1 unit per kilo insulin per hour	16	54	0.18	0.42	0.38
	17	67	0.21	0.29	0.54
	18	71	0.42	0.41	0.44
B. *Adreno-pancreatectomized cats*					
300 mgm. glucose + 1 unit per kilo insulin per hour	21	71	0.14	0.26	0.14
	22	56	0.27	0.32	0.17
	23	69	0.20	0.34	0.36
500 mgm. glucose + 1 unit per kilo insulin per hour	24	119	0.33	0.29	0.19
	25	121	0.15	0.36	0.58
	26	76	0.38	0.13	0.15
	27	106	0.43	0.29	0.13
600 mgm. glucose + 1 unit per kilo insulin per hour	28	90	0.20	0.45	0.25
	29	88	0.53	0.60	0.28
	30	76	0.20	0.60	0.32
	31	88	0.27	0.47	0.36

Animals were tested for periods of three, four or five hours. Heart glycogen figures quoted represent averages for apex, body and base samples of the organ.

glycogen in this highly important organ, even in the presence of more than adequate glucose supplies.

Insulin was tested on adrenalectomized animals in different concentrations and with different amounts of glucose. Frequently, in preliminary tests, severe convulsions appeared within an hour or so after injection, and smaller doses of insulin with relatively large amounts of glucose were then employed. It will be observed (tables 2 A, 3) that, in the absence

of the adrenals, no hepatic glycogenesis occurred under insulin action. This was true in both short-term experiments extending over a few hours, and long-term tests over several days. Small increases in skeletal muscle and cardiac glycogen are nevertheless indicated.

The ability of the cortico-adrenal hormone to bring about synthesis of glycogen in adrenalectomized animals was readily apparent in all experiments. It is necessary to refer only to the average figures obtained

TABLE 3

Carbohydrate values under different experimental conditions (cats)

CONDITION	TREATMENT	NO. OF ANIMALS USED	BLOOD SUGAR	LIVER GLYCOGEN	MUSCLE GLYCOGEN	HEART GLYCOGEN
			mgm. per cent	*per cent*	*per cent*	*per cent*
Normal	None	10	88	1.22	0.43	0.61
	Glucose-treated*	10	88	3.31	0.50	0.83
Adrenalectomized	Untreated	10	57	0.07	0.21	0.21
	Glucose-treated*	10	80	0.26	0.33	0.35
	C-A extract + glucose	6	151	1.77	0.54	0.66
	Insulin + glucose†	8	57	0.25	0.42	0.54
	Insulin + glucose‡	6	80	0.37	0.37	0.36
Pancreatectomized	Untreated	5	392	0.72	0.44	0.80
	Glucose-treated	5	317	0.99	0.36	0.85
	C-A extract + glucose	5	292	1.01	0.37	0.63
	Insulin + glucose	5	98	0.88	0.47	0.74
Adreno-pancreatectomized	Untreated	10	73	0.14	0.46	0.43
	Glucose-treated	6	224	0.25	0.38	0.45
	C-A extract + glucose	5	193	0.81	0.40	0.71
	Insulin + glucose	11	87	0.28	0.37	0.27

* Glucose solution orally (see details, table 1) for periods up to 6 days.

† Tests made for periods up to 5 hours (table 2).

‡ Tests for 2 and 3-day periods.

Glucose was given in 3 per cent solution, 1 per cent body weight per hour for 4 to 6 hours; 5 to 10 cc. of cortico-adrenal extract were given per hour. Note exceptions above and further details in text.

in this group (table 3). In some cases liver glycogen values up to 2 or even 3 per cent were found after cortico-adrenal treatment.

Pancreatectomy. Untreated pancreatectomized cats with symptoms of weakness showed, along with the usual hyperglycemia, very little change from the normal values for liver, muscle and heart glycogen. Glucose and glucose-insulin treated animals showed slightly augmented liver glycogen levels. Administration of cortico-adrenal extract with glucose

also resulted in increased liver glycogen concentration, and maintenance of the hyperglycemic condition (table 3). The relatively small changes in glycogen in the liver and muscle tissues of pancreatectomized animals under different conditions may be emphasized.

Adreno-pancreatectomized animals. When the adrenals and pancreas were removed in cats, it was particularly notable that the liver glycogen fell to a very low level, and the blood-glucose concentration also was sometimes below normal. In many respects the animals were similar to those which had been adrenalectomized; they developed a comatose state, much like that of adrenal insufficiency, and survived only a few days if untreated. An animal could be restored time after time from the prostrate condition, however, by cortico-adrenal extract; the blood sugar was raised by such injections, and life could be maintained for at least a week or two, and probably much longer if desired. Glucose solutions also restored adreno-pancreatectomized cats showing symptoms of insufficiency, but only temporarily, for periods of a few hours. The following protocols are illustrative:

Protocol: Adreno-pancreatectomy.
Cat 35. Weight 1.60 kilos, female.
April 11, 1939, 10:40 a.m., pancreas and both adrenals removed.
12, 10 a.m., blood sugar 84 mgm. per cent; animal weak; gave 15 cc. cortico-adrenal extract.
13, 9 a.m., blood sugar 200 mgm.; condition good; 10:00 a.m., 10 cc. extract; 11 p.m., 10 cc. extract.
14, 9 a.m., good condition; 10 a.m., blood sugar 238 mgm., 10 cc. extract; 12 noon, blood sugar 256 mgm.
15, normal.
16, normal; blood sugar 250 mgm.
17, 9:45 a.m., blood sugar 208 mgm.; good condition; gave 10 cc. extract; 11:45 a.m., blood sugar 247 mgm.; 1:45 p.m., blood sugar 250 mgm.; tested insulin effect, gave 1 unit per kilo; 4:15 p.m., 2 u.p.k. insulin; 6:10 p.m., blood sugar 95 mgm.; weak; 7:20 p.m., blood sugar 61 mgm.; cat dying; took final samples. Liver glycogen, 0.69 per cent; muscle glycogen, 0.48 per cent; heart glycogen, 0.81 per cent.

Protocol: Adreno-pancreatectomy.
Cat 36. Weight 1.80 kilos, male.
April 11, 1939, 10 a.m., adrenals and pancreas removed.
12, 9:50 a.m., blood sugar 57 mgm. per cent; cat weak; given 36 cc. 3 per cent glucose solution intraperitoneally (2 per cent body weight); 10:50 a.m., improved; repeated glucose injection; 12:05 p.m., blood sugar 255 mgm.; 12:45 p.m., weak again; gave 15 cc. cortico-adrenal extract in 0.9 per cent saline, i.p.; 1:45 p.m., blood sugar 244 mgm.; slight improvement; 10 cc. extract; 5 p.m., much improved; 10 cc. extract; 10 p.m., condition normal.
13, 9 a.m., blood sugar 333 mgm., normal condition; 9 p.m., 10 cc. extract.
14, 10 a.m., blood sugar 263 mgm.; normal; 10 cc. extract; 10 p.m., condition good, no extract.

15, 8:30 a.m., blood sugar 250 mgm.; cat normal; no treatment; 9:45 p.m., same condition.
16, 7:45 a.m., blood sugar 200 mgm.; condition good; 6 p.m., blood sugar 190 mgm., slightly weak, gave 10 cc. extract; 7 p.m., blood sugar 222 mgm.; cat much stronger.
17, 9:45 a.m., blood sugar 220 mgm.; good condition; 10 cc. extract; 4:45 p.m., blood sugar 278 mgm.; 1:40 p.m., blood sugar 266 mgm.; tested insulin action, 1 unit per kilo; 4:15 p.m., blood sugar 250 mgm.; condition normal; gave insulin 2 u.p.k.; 6:10 p.m., blood sugar 86 mgm., cat weak; 7:20 p.m., blood sugar 54 mgm.; animal apparently dying. Took final tissue samples: liver glycogen, 1.23 per cent; muscle glycogen, 0.61 per cent; heart, 0.64 per cent.

While the blood sugar was raised in adreno-pancreatectomized animals by glucose injection, no significant changes in glycogen levels were observed. Furthermore, insulin given along with glucose was unable to influence the liver, muscle and cardiac glycogen values. Various amounts of glucose with insulin were used without positive effect (table 2, B). Adreno-pancreatectomized cats were markedly hypersensitive to insulin injections, it may be noted, and after many tests small doses only were utilized. The pancreatic hormone did not restore or alleviate the condition of insufficiency observed in these animals (see protocols).

Cortico-adrenal extract given with glucose affected notably the liver and cardiac glycogen in adreno-pancreatectomized cats, raising both to approximately normal concentrations (table 3).

Discussion. The present paper together with the preceding (Corey and Britton, 1940) confirm and amplify earlier work from this laboratory emphasizing the rôle of the adrenal cortex in carbohydrate regulation. For several years after the results of the senior author and his colleagues had been presented (1930 onwards), there ensued vigorous discussion regarding the validity of their observations. Swingle (1933), Parkins *et al.* (1936), Grollman and others offered opposing evidence. In an extended monograph and again in a later paper (1936, 1938), Grollman affirmed that the cortico-adrenal hormone did not affect carbohydrate metabolism. Attention at this time turned more particularly to the electrolyte disturbances in adrenal insufficiency.

Recently, with the greater availability of potent extracts of the adrenal cortex as well as synthetic preparations, emphasis has again been given to the possible influence of the adrenal cortex on carbohydrate metabolism. Many investigators have now confirmed our earlier results, and attached greater importance to the carbohydrate complications in experimentally produced adrenal insufficiency as well as in Addison's disease. The numerous experiments of Long and his colleagues (1940), and Verzàr (1939) are of particular note. Anderson et al. (1939) observed that the blood sugar and liver and muscle glycogen were all reduced as early as 24

hours after adrenalectomy in the rat. Uyldert and co-workers (1939) found further in the dog that before the clinical symptoms of adrenal insufficiency appear, there occurs a decrease in the amount of sugar in the blood.

The striking ability of cortico-adrenal extract to raise tissue glycogen levels in adrenalectomized, pancreatectomized and adreno-pancreatectomized animals is demonstrated in the present paper. This sets the adrenal cortex apart in a distinct class as a glycogenetic factor. Insulin in contrast showed relatively little effect, and even in pancreatectomized animals it did not raise the liver glycogen to levels equal to those produced by cortico-adrenal extract. There was evidence in a few cases, however, that insulin brought about some increase in muscle glycogen. A consideration of

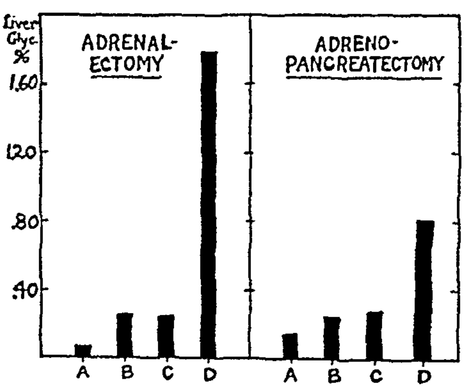

Fig. 1

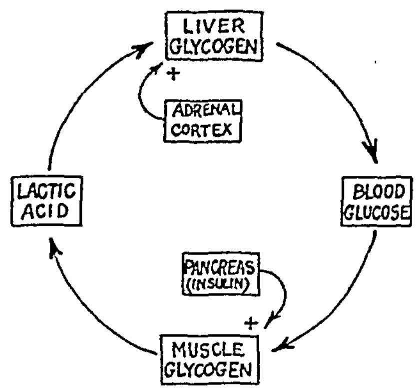

Fig. 2

Fig. 1. Left. Hepatic glycogen levels in adrenalectomized and adreno-pancreatectomized cats under different conditions. A, untreated; B, glucose-treated; C, insulin plus glucose; D, cortico-adrenal extract plus glucose. The marked action of the adrenal hormone in elevating liver glycogen is apparent.

Fig. 2. Right. Diagram indicating the preponderating effects of the cortico-adrenal and pancreatic hormones on the carbohydrate cycle.

glycogen levels in adrenalectomized and adreno-pancreatectomized cats in different experimental tests, shown in the accompanying graph (fig. 1), demonstrates the preponderating influence of the cortical hormone on hepatic glycogen levels.

The restoration by cortico-adrenal extract of adrenalectomized or adreno-pancreatectomized animals showing symptoms of insufficiency would appear to be dependent, at least in part, on an increase in circulating blood glucose. The injection of glucose solution alone was also temporarily effective in both operated groups, while insulin was of no avail.

That the adreno-pancreatectomized animal deteriorates primarily because of lack of cortico-adrenal tissue appears evident. Most of the symptoms which are observed a few days after operation are similar to

those found in adrenal insufficiency, and the carbohydrate losses strongly indicate the dominant effect of cortical removal.

There is still a great deal of confusion regarding insulin action in the body, although it appears an accepted fact that augmented oxidation of carbohydrates occurs under its influence. In Macleod's text (1938) it is observed that in normal (as well as diabetic) animals, "carbohydrate storage" is promoted by insulin injection; Wiggers (1939) notes that this hormone increases glycogen deposition in muscle; and Best and Taylor (1939) remark that liver glycogen is increased by insulin in normal rabbits, probably as a secondary effect due to adrenalin liberation.

In a recent review, Cori (1940) states that insulin is able to effect glycogen formation in liver and muscle, but is not indispensable for the reaction glucose → glycogen. No mention is made of any influence of the adrenal cortex, although one section is devoted to the "effect of hormones on carbohydrate metabolism." Soskin (1940) remarks in another review on carbohydrate metabolism that "it may be supposed . . . that the endocrine (carbohydrate) balance consists of the opposing influence of the hormones of the pancreas and of the anterior hypophysis."

Although insulin has in the past been given the chief place in discussions of carbohydrate metabolism, it is rather amazing that little or no attention has been given to the adrenal cortex in such articles as the above. In contrast Kendall (1940), in discussing the adrenal cortex, placed at the head of its list of functions the regulation of carbohydrate metabolism. A key position for the adrenal cortex in the carbohydrate cycle is indicated (fig. 2) by the results given herein and those we have advanced for many years past.

SUMMARY

The influence of the adrenal cortex is highly important in regulating carbohydrate levels in the body. Comparison of blood sugar and liver, skeletal muscle and heart glycogen concentrations in adrenalectomized, pancreatectomized and adreno-pancreatectomized cats under different conditions points directly to this conclusion. The effects of cortico-adrenal extract and insulin in conjunction with glucose administration have been tested in all series.

Adrenalectomized cats show very severe losses in blood glucose and liver, muscle and cardiac glycogen when untreated. They are able to form only slight amounts of glycogen from glucose given by mouth over periods up to 6 days. No increase in liver glycogen occurs when insulin is given with glucose, but small rises in muscle and cardiac glycogen may occur. Large increases in blood glucose and all glycogen values are produced by extracts of the cortex used with glucose.

Pancreatectomized animals when untreated show practically normal

amounts of liver, skeletal and cardiac muscle glycogen almost up to the time of death. They are able to form liver glycogen from glucose solution alone. Comparatively, somewhat more muscle glycogen appears to be deposited under the influence of insulin, and slightly more liver glycogen under cortico-adrenal extract.

Adreno-pancreatectomized cats which are untreated often show low blood sugar and liver glycogen levels when symptoms appear, somewhat similar to the conditions observed in adrenal insufficiency. They may be restored from severe symptoms time after time, and life may be greatly prolonged, by the injection of cortico-adrenal extract. Such resuscitation is accompanied by an increase in blood sugar. Glucose also brings about temporary restoration. Administration of glucose solution alone or with insulin does not affect significantly the formation of glycogen in adreno-pancreatectomized animals. Cortico-adrenal extract given with glucose brings about large deposits of glycogen in the liver and cardiac tissues.

Cortico-adrenal extract is observed to be well able to form liver glycogen in the absence of the pancreas; insulin does not do so in animals following loss of the adrenal cortex. Apparently the carbohydrate hormone of the cortex stimulates markedly hepatic glycogenesis, while insulin may affect favorably the formation of glycogen in muscle.

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OBSERVATIONS CONCERNING THE PRESSOR SUBSTANCE PRESENT IN THE ISCHEMIC KIDNEY BLOOD OF THE DOG

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Although many advances have been made in the study of arterial hypertension of renal origin since the pioneering studies of Goldblatt and his associates (1), the exact nature of the mechanism that underlies the elevation of blood pressure following the production of renal ischemia is still unknown. It seems that renal ischemia produces an elevation of blood pressure which is independent of primary changes in the nervous system (2, 3, 4, 5), the heart (6), the circulating blood volume (7), and the excretory efficiency of the kidney (8).

The work of Houssay and his associates (9) demonstrated clearly that the ischemic kidney produced a humoral agent capable of raising the blood pressure of the dog. The presence of renin in the venous blood of both the isolated kidney perfused under reduced pulse pressure and the intact, partially ischemic kidney was reported by Page and his associates (10, 11). A pressor substance was reported by Taquini (12) and by Prinzmetal and his associates (13) to be present in the completely ischemic kidney. It has just come to our attention that Braun-Menendez and his associates (14) have reported the presence of a pressor substance in the venous blood of partially ischemic dogs' kidneys which they believed was identical to the substance described as "hypertensin" by Taquini. However, this last substance is not renin but a product resulting from the reaction between renin and a pseudoglobulin of normal blood. Its chemical, pharmacological and physiological properties appear identical to those of the substance "angiotonin" described by Page and Helmer (15).

In the present communication, the presence of a pressor substance in the venous blood of both the isolated perfused kidney and the partially ischemic but intact kidney of the hypertensive dog is reported. Further, some of the properties of the pressor substance found in this ischemic kidney blood are described. Although the pressor substance found to be present in the ischemic kidney blood of our experiments has many properties similar to those ascribed to "hypertensin" and its apparent counterpart, "angiotonin", it nevertheless does not appear to us to be identical to either.

Throughout the remainder of this report, frequent references will be made to the renal pressor substance, but it must be emphasized that this pressor quality of ischemic kidney blood may be due to a mixture of several substances. Also, in this report the terms renal pressor substance and ischemic kidney blood will be used interchangeably because the pressor constituent of our samples of ischemic kidney blood has not yet been isolated from the blood plasma.

I. THE PRESSOR SUBSTANCE PRESENT IN RENAL BLOOD PERFUSATE. *Methods:* The isolated, freshly removed kidney of a normal dog was perfused with 500 to 700 cc. of normal blood (heparinized) obtained from the femoral artery of another normal dog. A constant nitrogen escape apparatus was used which effected a constant, non-pulsatile pressure upon the reservoir in which the blood to be perfused was contained.

TABLE 1

Pressor effect of perfused blood

EXPERIMENT NUMBER	PERFUSION OF KIDNEY AND SPLEEN WITH BLOOD						EFFECT OF PERFUSED KIDNEY AND SPLEEN BLOOD ON B.P. OF NEPHRECTOMIZED DOG			
	Organ perfused	Total blood perfused	Renal flow	Duration of perfusion	Number of circulations	Perfusion pressure	Total perfused blood given	Effect of perfused blood on B.P.	Time interval before B.P. change	Duration of B.P. change after injection of perfused blood
		cc.	*cc./min.*	*min.*		*mm. Hg*	*cc.*	*mm. Hg*	*min.*	*min.*
1-A	Kidney	600	9.0	233	2	145	150	+60	<1	19
2-B	Kidney	600	5.9	167	2	170	120	+85	<1	23
3-C	Kidney	350	3.5	83	1	145	75	+45	<1	20
4-D	Kidney	500	6.0	95	2	120	80	+30	<1	10
5-E	Kidney	500	3.0	25	1	150	80	+30	<1	24
Average......		510	5.48	120.6	1.6	146	101	+50	<1	19.2
6-F	Spleen	500	7.75	142	3	120	100	+8	4-5	<1
7-G	Spleen	500	2.5	53	1	130	50	−10	2-3	<1

The isolated kidney to be used for the perfusion was always immersed in a saline water bath maintained at 37°C., immediately after its removal from a living dog. The renal artery was connected by a cannula to the pressure reservoir containing the arterial blood, and the renal vein was cannulated for the collection of the venous blood. A wide and shallow pan was used for the collection of the venous blood. This pan was constantly but gently agitated to ensure a maximum aeration of the blood before it was again returned to the pressure system for recirculation through the kidney. The entire perfusion apparatus was so designed that the

recirculation of any sample of perfusate could be done without any interruption of the maintenance of a constant pressure. The rate of renal flow, the duration of the perfusion, and the perfusion pressures maintained are given in table 1. Five perfusions were performed.

After the completion of the renal perfusion, samples of perfusate (75–150 cc.) were given at the rate of 10 cc. per minute by vein to nephrectomized dogs (10–15 kgm.) maintained under anesthesia with pentobarbital sodium. In the recipient dog, the mean blood pressure was determined by cannulation of the right carotid artery. Before the introduction of the renal blood perfusate, a control infusion of normal blood equal in

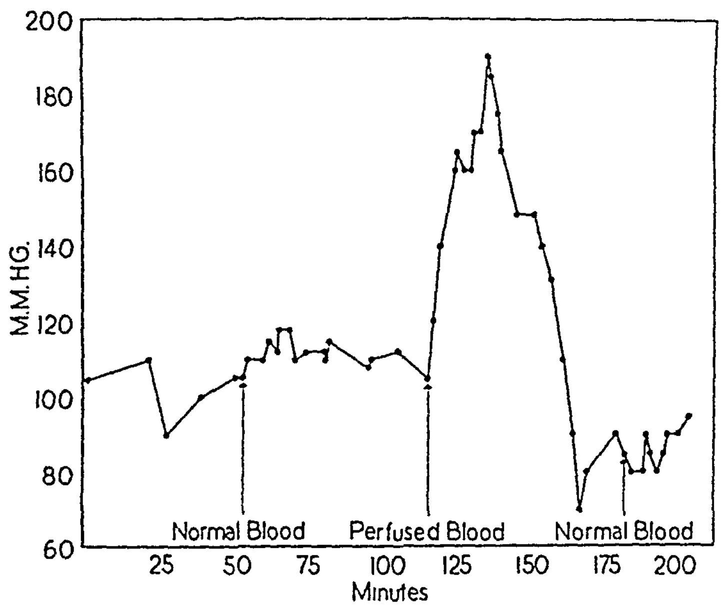

Fig. 1 (Expt. 2-B). The effect of perfused renal blood on the blood pressure of a nephrectomized animal is shown. The effect of the same blood before renal perfusion is also shown. It was given both before and after the introduction of perfused renal blood. One hundred twenty cubic centimeters introduced within 12 minutes were given in each administration.

quantity to the perfusate to be injected was first given. Two blood perfusions of isolated spleens were also performed.

Results: The employment of a constant perfusion pressure caused a marked reduction in renal blood flow in the isolated kidney (see table 1), despite the fact that the average perfusion pressure in the five experiments was 145 mm. Hg.

The blood perfused through the isolated kidney in the manner described above exhibited a strongly pressor effect when given to a nephrectomized dog in contrast to the negligible effect observed when equal quantities of normal blood were given. Thus (see table 1 and fig. 1), in five experiments, the average administration of 101 cc. of renal blood perfusate provoked a

rise of 50 mm. of Hg in the blood pressure of the recipient dog. The pressor effect began in less than one minute and lasted for over 19 minutes in the five experiments. In one experiment (5-E), the perfusion was maintained for only 25 minutes and the blood was not recirculated. Nevertheless, the blood sample contained the pressor substance noted in the other four perfusates. Finally, it may be observed that blood perfused through the spleen in the same manner as described above produced no significant pressure rise when given to a recipient dog.

II. The pressor substance present in the ischemic kidney blood of the dog. Because it was observed that blood obtained from a kidney perfused with normal arterial blood for as little as 25 minutes possessed a pressor effect, it was thought advisable to observe the effects of renal blood leaving intact kidneys with a considerable reduction in renal artery flow upon the blood pressure of the same dog and also upon another dog.

Methods: In these experiments, dogs were anesthetized with pentobarbital sodium and before any other procedure was executed, blood pressure readings were taken in the manner described above until a stable control level had been reached. Then each kidney was isolated, a Goldblatt clamp was attached, tightened to complete obstruction and then released sufficiently to permit a small blood flow to the kidney which was always determined by direct inspection. After the adjustment of the clamps, the incisions were rapidly closed and the pressure of the dogs was recorded continuously for the next 4 to 5 hours. In three dogs one kidney was removed and the kidney remaining was clamped as described above.

After periods of time varying from 172 to 338 minutes, the dogs were given 4 to 5 cc. of purified heparin (Connaught) and reoperated. The renal vein of one ischemic kidney was then cannulated and the flow was collected and measured. The rate of flow varied from 10 to 20 cc. per minute and the average time taken for the collection of the renal venous blood was 45 minutes, and about 500 to 600 cc. of blood were obtained in a single experiment. Samples of this blood were then given to previously nephrectomized dogs. Control infusions of normal blood were given to the recipient dogs both before and after the infusion of the blood from the partially ischemic kidney. Nine experiments were performed.

Results: The pressor effect of acute partial renal ischemia on the same dog. Of the nine dogs whose kidneys or kidney (the other being removed) were made partially ischemic, seven exhibited a fairly immediate rise in their blood pressures varying from 15 to 40 mm. Hg above the control pressure level (see table 2 and fig. 2). In the two dogs (3-L, 8-A) not showing a rise, considerable hemorrhage was observed to have occurred.

The pressor effect of blood from a partially ischemic kidney on the nephrectomized dog. As can be seen in table 2, the ischemic kidney blood when

given intravenously to nephrectomized dogs (10–15 kgm.) effected a rapid rise of blood pressure in each of the nine experiments, the average rise being 26 mm. of Hg. Further, the ischemic kidney blood was similar to the renal blood perfusate in the rapidity and duration of its action. The greater rise following the introduction of the renal blood perfusate may well be due to the larger amounts of blood given and not due to any essential difference in the pressor substance contained in the blood samples. The introduction of normal blood into the nephrectomized dogs in quantities and at infusion rates comparable to those used in the introduction of partially ischemic kidney blood did not provoke a significant blood pressure change in the recipient.

TABLE 2

Pressor effect of blood from partially ischemic kidney

DOG NUMBER	PARTIAL ISCHEMIA OF KIDNEY IN DOGS AND COLLECTION OF ISCHEMIC KIDNEY BLOOD							EFFECT OF ISCHEMIC RENAL BLOOD ON B.P. OF NEPHRECTOMIZED DOG			
	Release of left clamp	Release of right clamp	Maximum B.P. rise in a dog with ischemic kidneys	Time for beginning of rise	Duration of partial ischemia before renal flow collection	Rate of renal flow	Duration of renal flow collection	Total of ischemic renal blood given	Effect of ischemic renal blood on B.P.	Time interval before B.P. change	Duration of B.P. change after injection of ischemic renal blood
	turns	*turns*	*mm. Hg*	*min.*	*min.*	*cc./min.*	*min.*	*cc.*	*mm. Hg*	*min.*	*min.*
1-J*			+40	9	220	11	69	70	+20	<1	27
2-P			+40	106	248	19.7					
3-L†			0		198						
4-M	½	¾	+15	190	338	9.8	52	100	+30	<1	11
5-S	¼	0	+22	85	172	15	34	55	+30	<1	17
6-V	⅛	¼	+20		240	6.1	40	100	+12	<1	1§
7-W*	¼		+23	22	224	12.2	30	50	+27	<1	23
8-A‡	⅜	¼	0		213	12.0	60	60	+23	<1	21
9-B	⅜	⅜	+25	250	266	11.8	75	50	+40	<1	10
Average..........			20.5	110.3	235.4	12.2	45	60.6	+26	<1	15.7

* One kidney removed.

† Dog had hemorrhage.

‡ Dog went into profound shock.

§ Recipient dog was not bilaterally nephrectomized.

The pressor properties of ischemic kidney blood were compared with those of renin in a series of 13 nephrectomized dogs. In the seven dogs receiving an average injection of 2.5 mgm. of purified renin there was an average rise in their blood pressures of 40 mm. Hg. The maximum elevation of pressure occurred six minutes after the injection of renin and the pressor effect remained for over 29 minutes. The average introduction of

53 cc. of ischemic kidney blood into each of six dogs led to an average blood pressure rise of 36 mm. Hg which was comparable to that following the injection of renin. Unlike renin, however, the ischemic kidney blood exerted its maximum pressor effect within one minute and subsided entirely in 13 minutes. The differences in the effect of these two substances cannot be explained upon a quantitative basis and it must be considered that the two substances are not completely identical.

The induction of renin tachyphylaxis in four nephrectomized dogs made the dogs unresponsive to the administration of ischemic kidney blood which prior to the induction of tachyphylaxis evoked a typical pressor response in these dogs. However, when two dogs were made insensitive to ischemic kidney blood by repeated injections of the latter, the injection of renin (1 mgm.) still provoked a pressor response which was comparable

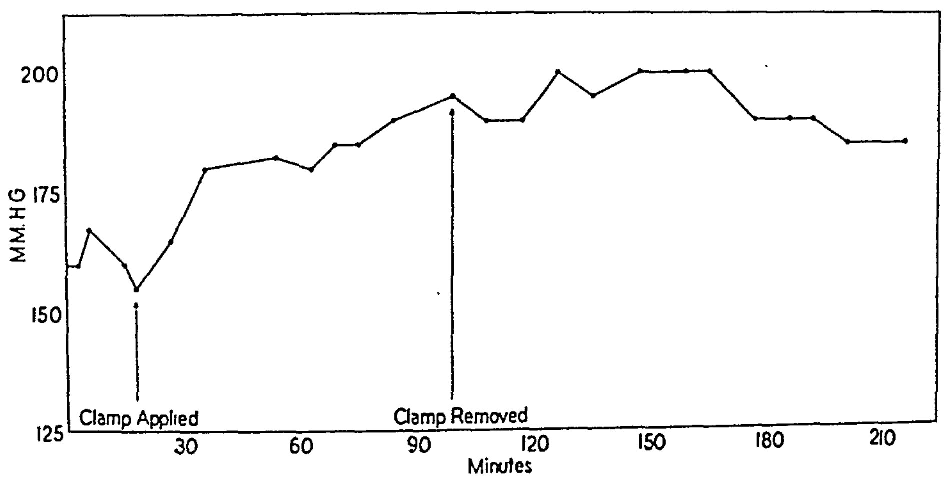

Fig. 2 (Expt. 1-J). The acute effect of unilateral partial renal ischemia of one remaining kidney on the blood pressure of a dog.

to that obtained before the induction of the ischemic kidney blood tachyphylaxis.

Properties of the pressor substance in ischemic kidney blood. 1. *The effect of heat.* In previous preliminary experiments it was found that the renal pressor substance was present in the plasma fraction of ischemic kidney blood. When four different samples of ischemic kidney blood containing the renal pressor substance were heated at 60°C. for 10 minutes, the typical pressor response was lost. For the heated ischemic kidney blood plasma produced a rapid, shortlasting rise (not over 2 min. in duration) in the blood pressure of the recipient dog, and this evanescent rise was followed by a prolonged decline in the blood pressure below the preinjection level. Normal plasma samples, when heated, produced this same type of pressor response when given to a recipient dog.

2. *The effect of dialysis upon ischemic kidney blood plasma.* Seven sam-

ples of heparinized plasma samples (25–50 cc.) obtained from ischemic kidney bloods, and five samples of normal plasma were dialyzed in cellophane bags against running water for 24 hours. At the end of this time, the contents of the bag were tested for pressor activity on the nephrectomized dog. It was found that there was a pressor response in the recipient dog following the administration of six of the seven dialyzed ischemic kidney blood plasma samples which averaged 32 mm. Hg, but there was no pressor response following the introduction of four of the five normal dialyzed plasma samples, and a rise of 10 mm. Hg. in the fifth sample. The pressor quality of the ischemic kidney blood plasma (dialyzed) was similar to that of the original ischemic kidney blood plasma both in the rapidity, intensity, and duration of the pressor effect.

3. *The effect of cocaine upon the the pressor action of ischemic kidney blood.* The prior intravenous administration of cocaine (20 mgm.) in four nephrectomized dogs did not prevent or diminish the pressor response following the administration of 50 cc. of ischemic kidney blood. The average pressor response following the introduction of the ischemic kidney blood (50 cc.) was 30 mm. Hg before and 38 mm. Hg after the administration of the cocaine in the four dogs observed.

III. The length of time necessary for the production of the renal pressor substance following partial renal ischemia. In the second series of experiments partial renal ischemia was maintained for about four hours, but the time actually necessary for the production of the renal pressor substance was not known. Accordingly, the following experiments were performed.

Methods: The renal artery and vein of the normal anesthetized dog were isolated as described before. The Goldblatt clamp was applied, and the renal artery was severely constricted after 3 to 5 cc. of purified heparin had been given to the dog. The renal vein was then cannulated and the venous blood immediately collected in 15 minute samples at a maintained rate (by adjustment of the Goldblatt clamp) of 5 to 10 cc. per minute. At the end of an hour, the collections were discontinued. Four experiments were performed.

Fifty cubic centimeters of each 15 minute collection were then injected into the femoral vein of an anesthetized, previously nephrectomized dog (10–15 kgm.) and the pressor response was noted. It was found that the introduction of a similar quantity of either saline solution or normal dog's blood at the rate of injection used (25 cc/min.) did not cause a significant pressor response in the recipient dog.

Results: In the four experiments, it was observed that in each of the dogs renal venous blood collected during the first 15 minutes contained sufficient pressor material to evoke an average pressure rise of 35 mm. Hg when 50 cc. of it were given to another dog. Fifty cubic centimeter

quantities of the second, third and fourth 15 minute collections evoked average pressor responses of 35, 35 and 42 mm. Hg respectively in recipient dogs. Thus, the pressor potency of the first 15 minute collection of ischemic kidney blood was comparable not only to that of the later blood collections but also to that of venous blood collected after four hours of partial renal ischemia (see table 2).

IV. NEUTRALIZATION OF THE RENAL PRESSOR SUBSTANCE BY THE NORMAL DOG'S KIDNEY. Several observers (16, 17) have noted that the hypertension following the production of partial renal ischemia in the dog may be abolished or masked by the presence of a normally functioning kidney.

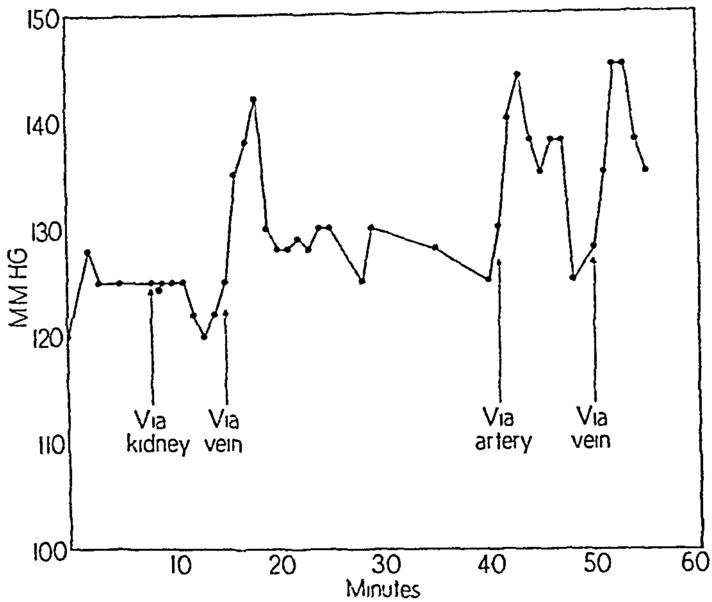

Fig. 3 (Expt. 13-A). The effect of the normal kidney on the pressor action of ischemic kidney blood. Fifty cubic centimeters of ischemic kidney blood given to recipient dog via—1, renal artery, 2, femoral vein, 3, femoral artery, and 4, femoral vein again.

Therefore it was thought advisable to determine the action of the intact and normally functioning kidney on the renal pressor substance.

Methods: The left renal artery of the normal, anesthetized dog was temporarily clamped in two places and cut between the clamps after 3 to 5 cc. of heparin had been given. Then, the cut ends of the artery were ligated around two barrels of a small three-barreled T-shaped cannula. The third barrel of the cannula was attached to a narrow, flexible rubber tube extending through the abdominal wall of the dog. This tube was temporarily clamped with a hemostat and then the clamps on the renal artery were released. The wound was closed and both the left femoral artery and vein were similarly cannulated. Thus, ischemic kidney blood could be injected by syringe into the left renal artery, the femoral artery

and the femoral vein of the same dog. Two minutes were taken for each injection. Seven such experiments were performed.

Results: In the seven experiments (see fig. 3), a marked difference was observed in the response of the same recipient dog to the same quantity of renal pressor substance when it was given via the renal artery and when it was given via the femoral artery or vein. For when 40 cc. of ischemic kidney blood were given via the renal artery, the average pressor response was 5 mm. Hg, (range 0–10 mm. Hg), whereas the introduction of an equal quantity of the same lot of ischemic kidney blood into the femoral artery and vein led to an average rise of 16 and 18 mm. Hg, respectively. It may be mentioned that in one experiment the introduction of the renal pressor substance via the splenic artery led to a well-marked pressure rise comparable in extent to that found when the substance was injected into the femoral vein.

SUMMARY AND CONCLUSIONS

In the preceding observations the presence of a pressor substance in the venous blood of either an isolated perfused kidney or of the intact kidney of the dog is reported. It was found that this substance could be detected in the blood leaving the partially ischemic kidney within 15 minutes after the initiation of the partial ischemia. Further, it was found that its pressor quality per cubic centimeter at this time was as strong as that found in blood obtained after a much longer period of partial ischemia. This last observation indicates that the production of this substance is not necessarily dependent upon progressive autolysis or destruction of the kidney.

The ischemic kidney blood was found to have a pressor effect which differed from that of renin in that its action was immediate and of moderate duration. Further, animals made tachyphylactic to the introduction of ischemic kidney blood still reacted to the injection of renin. On the basis of these differences, it was felt that the pressor quality of the ischemic kidney blood was not due to the presence of renin alone.

The renal pressor substance was found to be nullified by the action of heat on the plasma containing it. It was also ineffective when injected into a dog via the renal artery supplying a normal kidney. However, the renal pressor substance could not be removed from ischemic blood plasma by prolonged dialysis of the latter nor was it ineffective when given to cocainized dogs.

Since this ischemic kidney blood contains a pressor substance that is not exactly similar to renin in its physiological actions and differs somewhat from "angiotonin" ("hypertensin") in some of its chemical properties, it is believed that the substance is not identical to either of them.

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